Appendix A
Center for Freshwater Biology Standard Operating Procedures (SOPs)

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Additional staff includes undergraduate and graduate students that serve as lab and field technicians and data entry technicians.

A.1. General Laboratory Protocols

A.1.1-Washing Bottles

Color Bottles (60ml opaque HDPE bottles) – Wash three times with DDI H_2O and let dry completely before capping them. Make sure the bottles and caps are face down when drying to assure dust particles do not settle inside and contaminate them. If contamination is suspected, rewash the bottles as above.

pH/Alk bottles (250ml amber HDPE bottles or PE syringes) – Rinse three times with distilled water (from the tap)and give a final rinse with DDI H_2O. Make sure the bottles, caps syringes and plungers dry completely before capping them and always let them dry face down to avoid contamination.

Chlorophyll bottles (2 and 3 liter Amber bottles) – Rinse three times with tap distilled water and give a final rinse with DDI H_2O. Place both the caps and bottles face down to dry completely before capping them.

Total Nutrient Bottles (250ml, 500 and 1000ml opaque HDPE Acid Washed Bottles) – These bottles should be rinsed once with DDI H_2O and then placed in the acid bath (30% HCL) for ten minutes (make sure the bottles are completely submersed). After 10 minutes, drain the acid from the bottles and rinse three times with DDI H_2O and place in the chicken wire drying rack. Always have both the bottles and caps face down to dry. Under no circumstances should these bottles be dried on the peg drying racks (this will contaminate the bottles and they will have to be rewashed). Once dry cap the bottles being careful not to touch inside the caps or inside the bottles. If there is any suspicion of contamination, rewash the bottles.

Dissolved Nutrient Bottles (60ml, 120ml and 250ml Amber HDPE) – These bottles should be rinsed once with DDI H_2O and then placed in the acid bath (30% HCL) for ten minutes (make sure the bottles are completely submersed). After 10 minutes, drain the acid from the bottles and rinse six times with DDI H_2O and place in the chicken wire drying rack. Always have both the bottles and caps face down to dry. Under no circumstances should these bottles be dried on the peg drying racks (this will contaminate the bottles and they will have to be rewashed). Once dry cap the bottles being careful not to touch inside the caps or inside the bottles. If there is any suspicion of contamination, rewash the bottles.

Zooplankton Bottles (500 ml opaque HDPE wide mouth bottles)- Rinse three times with tap distilled water and give a final rinse with DDI H_2O. Making sure no particulates are attached inside the bottle or cap. Place both the caps and bottles face down to dry completely before capping them.

Microbiological Bottles: (250 ml opaque wide mouth bottles)- Rinse with tap distilled water. Loosely cap and cover cap with the sterilization paper and rubber band the paper over the cap so that the cap and shoulder of the bottle are protected. Autoclave, carefully tighten cap without disturbing protective paper cover and store upright in the marked cabinet.

A.1.2- Mixing Commonly Used Reagents

5N Sulfuric Acid - In a 500 ml volumetric flask, add 350 ml DDI H_2O and place in an ice bath. Once cool add 70 ml concentrated H_2SO_4 and swirl the flask to mix the reagent and let cool. **(note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Once cool, slowly add DDI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DDI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm.

11N Sulfuric Acid - In a 500 ml volumetric flask, add 300 ml DDI H_2O and place in an ice bath. Once cool slowly add 155 ml concentrated H_2SO_4 and swirl the flask to mix the reagent. **(note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Slowly bring the solution to 500 ml and once cool remove from the ice bath. Once cool, slowly add DDI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DDI H_2O until a volume of 500 ml is reached, then cap the flask with Parafilm and mix gently.

10N Sodium Hydroxide - In a 500 ml volumetric flask, add 250 ml DDI H_2O and place in an ice bath on the magnetic stirrer (a white stirrer should be place into the flask at this time). Once cool slowly add 200 grams concentrated NaOH while the stirrer is spinning slowly. **(note: be sure to add the base slowly as the heat generated can cause the flask to break).** Once cool, slowly add DDI H_2O until the solution reaches the neck of the volumetric flask and remove the flask from the ice bath. When the volumetric flask reaches room temperature, add DDI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm.

Phenolphthalein indicator - In a 100 ml volumetric flask, dissolve 1 gram phenolphthalein crystals in 100ml 95% ethanol and store the solution in two 60 ml plastic drop dispensing bottles.

A.1.3- Analyzing Samples on the Milton Roy Model 1001+ Spectrophotometer

Turn the Milton Roy 1001+ spectrophotometer on at least 30 minutes prior to analyzing the samples. Then proceed as indicated below making sure you use the proper cuvettes when analyzing the respective samples. Setup the spectrophotometer as follows, inputting the appropriate numerical code for the desired analysis as detailed below:

1. Press the "Edit/Select" button (the spectrophotometer will scroll through a variety of options)
2. At the "3 Load Test" option press "Enter"
3. You will be prompted for a "Test No". Enter the proper numerical code from Table 1 below.

Table 1

Analyte	Code	Cuvette Type	Path Length	Blank
Chlorophyll a	23	Near UV Glass	5 cm	90% Acetone w/o $MgCO_3$
Total Phosphorus	25	Near UV Glass	10 cm	DDI H_2O
SRP	25	Near UV Glass	10 cm	DDI H_2O

4. Press "Enter"
5. You are now prompted to "Save Current" – press the "No" key
6. Press "Run/Stop"
7. You are now prompted to "insert blank" and "press enter"
   a) Place the appropriate blank into the appropriate cuvette (from the above table) and press the "enter" key. *Note: check the two cuvette faces for contamination (i.e. smears, dust) between samples and wipe the faces with Kimwipes if necessary.*
   b) The Milton Roy 1001^+ spectrophotometer will automatically zero itself and sequentially acknowledge "compensation complete" "insert sample" "press enter".
   c) You should now empty the contents of the cuvette into a waste container and pour the respective sample into the cuvette followed by the respective procedure outlined below.

A.1.3.a-Chlorophyll *a* – Blank the Milton Roy 1001^+ spectrophotometer with 90% acetone without $MgCO_3$ and record the absorption values when the spectrophotometer cycles through the second time. After the spectrophotometer is blanked discard the acetone into the waste acetone jar in the fume hood. Add the first chlorophyll *a* sample to the cuvette, place the cuvette into the spectrophotometer cell holder, close the lid and press the "Run/Stop" button. Record the second absorbency readings at 664nm and 750nm (the wavelengths will cycle through twice). Once you have recorded the 664nm and 750nm absorbencies, draw up 0.5ml of 0.1N HCl using a 0.5ml volumetric pipette and dispense the acid into your chlorophyll *a* sample. Cap the cuvette with the white caps and invert the cuvette 10 times or until the sample is no longer cloudy (the sample will remain cloudy if it is not well mixed). Place the cuvette into the cell chamber, close the lid and wait 90 seconds. After 90 seconds press the "Run/Stop" button. Record the second absorbency readings at 665nm and 750nm. After the sample is analyzed and all data have been recorded on the datasheet, dispose of the contents in the waste acetone container and rinse three times with 100% acetone (disposing off all acetone waste in the acetone waste bottle). Subsequent samples should be processed using the same procedure:

a) Pour the chlorophyll sample into the cuvette
b) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
c) Place the cuvette into the spectrophotometer cell holder.
d) Close the lid to the cell chamber.

e) Press "Run/Stop".
f) Record the 664nm and 750nm absorption values when the spectrophotometer cycles through the second time.
g) Remove the cuvette from the spectrophotometer, draw up 0.5ml of 0.1N HCl, and dispense the entire volume of hydrochloric acid into your chlorophyll *a* sample.
h) Cap the cuvette with the white caps and invert the cuvette 10 times or until the sample is no longer cloudy.
i) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
j) Place the cuvette into the spectrophotometer cell holder and close the lid.
k) Wait 90 seconds for the acid to convert the chlorophyll *a* to pheophytin.
l) Press "Run/Stop".
m) Record the 665nm and 750nm absorption values when the spectrophotometer cycles through the second time.
n) Discard the sample in the waste acetone container in the fume hood.
o) Rinse the cuvette three times with 100% acetone.
p) Repeat steps "a through h" until all samples have been processed and the data have been recorded. Note: you should analyze a 90% acetone blank without $MgCO_3$ after every eight samples and record the absorption values under the "spec check" column of the datasheet. A blank should always be the last sample analyzed and the results should be recorded on the datasheet.

Chlorophyll *a* standards – pre-packaged chlorophyll *a* should be analyzed on a monthly basis (the first week of each month) to assure the spectrophotometer is calibrated correctly. Pre-packaged chlorophyll samples should be purchased directly from the Turner Designs Company, catalog number 10-850, and consist of a low standard (approximately 15.5 µg/L) and a high standard (155 µg/L). The cost for these reagents is currently $175.00 and they should be purchased on a monthly basis and stored per the manufacturers specifications (@ -20^{o}C in the dark) to avoid chlorophyll degradation.

When analyzing standard reference materials (SRMs) you should assure the spectrophotometer has warmed up for 30 minutes followed by the series of steps detailed below. *Note: always check the two cuvette faces for contamination (i.e. smears, dust) between samples and after adding the HCL. Wipe the faces with Kimwipes as necessary.*

a) Press the "Edit/Select" button (the spectrophotometer will scroll through a variety of options.
b) At the "3 Load Test" option press "Enter".
c) You will be prompted for a "Test No". Enter 23 and press the "Enter" key.
d) You are now prompted to "Save Current" – press the "No" key.
e) Press "Run/Stop".
f) You are now prompted to "insert blank" and "press enter". Fill the chlorophyll cuvette with 90% Acetone without $MgCO_3$ and place the cuvette into the spectrophotometer cell holder.
g) The spectrophotometer will automatically zero itself and sequentially acknowledge "compensation complete" "insert sample" "press enter".
h) Discard the sample into the waste acetone container in the fume hood.
i) Break open the ampule that contains the 15.5 μg/L chlorophyll *a* standard.

j) Carefully pour the 15.5 μg/L chlorophyll *a* standard into the cuvette until the standard is flush with the neck (where the caps are placed). You have added approximately 15.0 ml of chlorophyll *a* standard.
k) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
l) Place the cuvette into the spectrophotometer cell holder.
m) Close the lid to the cell chamber.
n) Press "Run/Stop".
o) Record the 664nm and 750nm absorption values when the spectrophotometer cycles through the second time.
p) Remove the cuvette from the spectrophotometer, draw up 0.5ml of 0.1N HCl into a volumetric pipette, and dispense the entire volume of hydrochloric acid into your chlorophyll *a* sample.
q) Cap the cuvette with the white caps and invert the cuvette 10 times or until the sample is no longer cloudy.
r) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
s) Place the cuvette into the cell spectrophotometer cell holder and close the lid.
t) Wait 90 seconds for the acid to convert the chlorophyll *a* to pheophytin.
u) Press "Run/Stop".
v) Record the 665nm and 750nm absorption values when the spectrophotometer cycles through the second time.
w) Discard the sample in the waste acetone container under the fume hood.
x) Rinse the cuvette three times with 100% acetone.
y) Break open the ampule that contains the 155 μg/L chlorophyll *a* standard.
z) Carefully pour the 155 µg/L chlorophyll *a* standard into the cuvette until the standard is flush with the neck (where the caps are placed). You have added approximately 15.0 ml of chlorophyll *a* standard.
aa) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
bb) Place the cuvette into the spectrophotometer cell holder.
cc) Close the lid to the cell chamber.
dd) Press "Run/Stop".
ee) Record the 664nm and 750nm absorption values when the spectrophotometer cycles through the second time.
ff) Remove the cuvette from the spectrophotometer, draw up 0.5ml of 0.1N HCl into a volumetric pipette, and dispense the entire volume of hydrochloric acid into your chlorophyll *a* sample.
gg) Cap the cuvette with the white caps and invert the cuvette 10 times or until the sample is no longer cloudy.
hh) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
ii) Place the cuvette into the cell spectrophotometer cell holder and close the lid.
jj) Wait 90 seconds for the acid to convert the chlorophyll *a* to pheophytin.
kk) Press "Run/Stop".

ll) Record the 665nm and 750nm absorption values when the spectrophotometer cycles through the second time.
mm) Discard the sample in the waste acetone container under the fume hood.
nn) Rinse the cuvette three times with 100% acetone.

0.1N HCL Working Solution - *Note: the preparation of the HCl working solution should be undertaken under a fume hood designated for acid preparation.* Fill an acid washed 1000ml volumetric flask with approximately 500ml distilled de-ionized water (DDI H_2O) and place the flask into an ice bath in the fume hood for 15 minutes. Pour approximately 25 ml of 12.1N HCl (FS Cat # A144c-212) into a 50ml acid washed beaker under the fume hood. Draw up 8.3ml of the Stock HCl using a class-A glass pipette and dispense the acid into the 1000ml volumetric flask. Swirl the flask to obtain a homogeneous mixture. Place the 1000ml volumetric flask into a PP tray without ice and let the diluted acid solution come to room temperature. Once the diluted acid solution reaches room temperature bring the volumetric flask to volume by slowly adding DDI H_2O from a Nalgene squirt bottle. Parafilm the 1000ml volumetric flask and invert the flask 20 times to assure the 0.1N HCl solution is a homogeneous mixture. Store the 0.1N HCl solution in a PP tray on the lab bench.

A.1.3.b-Total Phosphorus & SRP - Blank the Milton Roy 1001^+ spectrophotometer with DDI H_2O and record the absorption values as the absorption values cycle through the second time. Pour out the water in a white one-gallon waste bucket. Subsequent samples should be processed by adhering to the following procedure:

a) Rinse the cuvette with the phosphorus sample you will analyze next.
b) Fill the cuvette with the phosphorus sample.
c) Check the cuvette faces and make sure they are clean. If necessary, wipe the faces with a Kimwipe.
d) Place the cuvette into the spectrophotometer cell holder.
e) Close the lid to the cell chamber.
f) Press “Run/Stop””.
g) Record the 660nm and 880nm absorption values when the spectrophotometer cycles through the second time.
h) Pour out the water sample in the white one-gallon waste bucket.
i) Rinse the cuvette three times with DDI H_2O and pour the waste in the white bucket between subsequent rinses.
j) Repeat Steps “a through h” until all phosphorus samples have been processed. Note: a DDI H_2O blank should be analyzed after every ten samples and the results should be recorded in the “spec check” column of the datasheet. A DDI H_2O blank should always be the last sample analyzed and the results should be recorded on the datasheet.

Written by Bob Craycraftand Jeff Schloss
Last Updated on 02/23/05
c:/CFBdocuments/SOPs/SOPL2005Spec.doc

A.2.- Soluble Reactive Phosphorus SOP (2005)

Reference Source: Standard Methods 20th Addition- 4500 P.E.

Sample Requirements: Non-acidified, filtered (0.45micron) sample

Note: use extreme caution when handling the phosphorus analytical glassware. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described in step three before proceeding any further.

1) Remove a maximum of 29 soluble reactive phosphorus samples from the freezer and let the samples warm to room temperature prior to proceeding to step five.

2) In the morning, mix 5N Sulfuric Acid (H_2SO_4) - In a 500 ml volumetric flask, add 350 ml DDI H_2O and place in an ice bath. Once cool add 70 ml concentrated H_2SO_4 and swirl the flask to mix the reagent. **(Note: be sure to add the acid slowly as the heat generated can cause the flask to break).** Place the heated flask into the ice bath and let the solution cool to room temperature. Once cool, slowly add DDI H_2O until the solution reaches the neck of the volumetric flask and place the flask into the ice bath for approximately 15 minutes. After the 15 minute cooing period, Parafilm the flask, invert the flask 10 times, and place the flask in a PP tray without ice until the solution reaches room temperature. Once the flask warms to room temperature, add DDI H_2O until a volume of 500 ml is reached and cap the flask with Parafilm. Invert the flask 10 times to obtain a homogeneous mixture, place the flask in a PP tray and store the flask on the laboratory bench until needed.

3) Acid wash (Place in the 30% Hydrochloric Acid (HCl) acid bath for 10 minutes):

84	125 ml Erlenmeyer flasks
1	50 ml graduated cylinder TD
1	100 ml graduated cylinder TD
1	250 ml graduated cylinder TD
6	500 ml volumetric flasks
1	1000 ml volumetric flask
1	100 ml beaker
1	10 ml graduated glass pipette TD
1ea.	0.5 ml, 2.0 ml, 2.5 ml, 5.0 ml, 10ml, and 25 ml class A volumetric pipettes

4) Transfer all pertinent information from the total phosphorus sample bottle to the soluble reactive phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).

5) Prepare sample flasks as follows:

Arrange the 125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 42B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples.

Prepare SRP standards and blank as follows (calibration curve):

Dilute pre-purchased HACH (cat #2059703) 3.00 mg/L stock standard, as 3.00 mg/L PO_4^{-3}, to the appropriate standard concentrations described in Table 2. *Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.* All dilutions will be performed by pipetting a fixed volume of stock standard, using class-A volumetric pipettes, into 500ML volumetric flasks that are labeled with the respective standard concentrations: 50 µg/L, 20 µg/L, 10 µg/L, 5 µg/L and 1 µg/L. All volumetric flasks are brought to volume with DDI H_2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved. *Note: If higher ranges are expected include a 100* µg/L *standard by diluting 50.0 ml in 500ml.*

Table 2. Orthophosphate working standards.

Standard Solution (*u* g P /L) [1]	HACH Stock Solution (ml) [2]	Erlenmyer Flask (ID) [3]
50	25.0	1
20	10.0	2
10	5.0	3, 13, 23, 33 and 41
5	2.5	4
1	0.5	5
(blank) 0	0.0	6 & 42

[1] Standards used to derive calibration curves (prepared within two hours of analysis). Note 3 µg PO4 = 1µg P

[2] volume of stock HACH standard (HACH cat # 2059703) added to a 500ML volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above.

[3] Erlenmeyer Flasks into which the respective standards and blanks are placed.

Pour out SRP standards as follows:

Invert the 500 ml volumetric flask containing the DDI H_2O blank and SRP standards three times immediately prior to pouring the reagents out. Rinse the 50 ml graduated cylinder three times with DDI H_2O. Measure 50 mls of each standard and pour the standards into the replicate Erlenmeyer flasks (50 mls into each flask) as denoted in Table 2. Between replicate samples (i.e. 1B and 2A) rinse out the graduated cylinder with 30% HCl and then rinse three times with DDI H_2O.

Pour out samples as follows:

Invert each sample twice and rinse the 50 ml graduated cylinder with the sample. Measure out 50 mls of water and pour into the replicate Erlenmeyer Flasks (A & B). Between replicate samples (i.e. 1B and 2A) rinse out the graduated cylinder with 30% HCl and then rinse three times with DDI H_2O. Note: Erlenmeyer flasks 12A and 12B will subsequently be spiked with phosphate standard (as described below) and should contain the same lakewater as was poured into flasks 11A and 11B. Likewise, flasks 32A and 32B will subsequently be spiked with phosphate standard and should contain the same lakewater as was poured into flasks 31A and 31B.

Spike flasks 12A, 12B, 32A and 32B as follows:
Pour out about 50ml pre-purchased (cat #2059703) 3.00 mg/L stock HACH standard, as 3.00 mg/L PO_4^{-3}, into an acid washed 100ml glass beaker. Using a 2.0 ml acid washed class-A volumetric pipette draw up a sample of the HACH standard into the pipette and dispense the standard into a waste bucket. Subsequently, draw up 2.0 ml of phosphate standard from the beaker and successively dispense 2.0 ml of standard into Erlenmeyer flasks 12A, 12B, 32A and 32B. This will constitute a phosphorus spike of 38.5 µg/L. Swirl each Erlenmeyer flask to obtain a homogeneous mixture.

6) Turn on the Milton Roy Spectronic 1001^+ at this time. The Milton Roy Spectronic 1001^+ should be on at least 30 minutes prior to running samples to assure stable readings.

7) Mix the following reagents:

Ammonium Molybdate	8.00 grams per 200 milliliters DI H_2O
Ascorbic Acid	5.28 grams per 300 milliliters DI H_2O
Potassium Antimonyl tartrate	0.28 grams per 100 milliliters DI H_2O

8) Mix the combined reagent:
The combined reagent should be mixed in a 1000 ml (acid washed) volumetric flask by measuring the volume of reagents in a graduated cylinder and adding the reagents in the following order (note: the reagents must be added in this order for the proper molecule to form and the graduated cylinder should be rinsed with DDI H_2O between the addition of each reagent); mixing the volumetric flask as each reagent is added:
   1) 500 mls 5N H_2SO_4
   2) 50 mls Antimony Potassium Tartrate
   3) 150 mls Ammonium Molybdate
   4) 300 mls Ascorbic Acid

Note: the mixed reagent is very unstable and should be made immediately prior to adding the reagent to the sample flasks.

9) Add the mixed reagent using the yellow and black pipetter (FS Cat# 13-681-25), rinsing the pipette by pipetting a sample of mixed reagent from the beaker and discarding it. After rinsing the pipette tip, add 8 mls of mixed reagent to each successive flask. As the mixed reagent is added a molecular complex (molybdenum blue) will form in the sample. The concentration of the molybdenum blue complex is proportional to the phosphorus concentration in the sample. While differences in color (low phosphorus concentrations) are not visible to the unaided eye, high phosphorus concentrations become various shades of blue; the bluer the sample the greater the phosphorus concentration.

10) Begin sample analysis 30 minutes after adding the mixed reagent to the first sample. See Procedures in Section A1.3 and A1.3.b for proper use of the spectrophotometer. Record the absorbencies at 660 and 880nm on the soluble reactive phosphorus data sheet. The spectrophotometer should be blanked with DDI H_2O and DDI H_2O blanks should also be run after every 10 flasks and should always be run after the final phosphorus sample has been

analyzed. Record the blank absorbencies (660 and 880nm) on the SRP datasheet in the "spec chk" column.

11) All phosphorus samples should be poured into a white (one-gallon) paint bucket and neutralized with baking soda prior to disposal.

12) Glassware cleanup - Immediately rinse out the flasks and other glassware three times with DDI H_2O after the run and place the glassware in the drying rack on the gray Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let sit for one hour, otherwise, fill the flasks with DDI H_2O and place out of the way until room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DI H_2O and place the glassware upside-down in the drying rack.

13) Calculating the SRP Coefficient from the calibration curve data:
After each analytical run enter the five initial SRP standards (samples 1-5) and the corresponding 880 nm absorption values into an Excel spreadsheet as depicted in the first two columns of table 3. Subtract the DI H_2O blank 880nm absorption value (sample 6) from each of the 880nm standard absorption values as depicted below. Regress the standards (dependent variable; Y) against the corrected 880nm absorption values (independent variable; X) to generate a statistical output as displayed below. You will now multiply your corrected 880nm absorption values by the X variable (SRP coefficient) to calculate your SRP concentrations (Table 3). As you can see, the prepared SRP standards compare well with the calculated SRP values. You will now use your SRP coefficient and the corrected 880nm absorption values of your samples (unknowns) to determine the remaining SRP concentrations. You should record your SRP coefficient, your DI H_2O blank (corrective factor) and your r^2 value on the back of your Orthophosphate (SRP) datasheet. Save the Excel spreadsheet as {date as YYYY/MM/DD}SRP.xls

Table 3: SRP Calibration curve*

SRP Standard (ppb)	880nm abs	880 nm abs (corrected for blank)	* Calculated SRP (ppb)
100	0.505	0.501	99.2
50	0.262	0.258	51.2
25	0.136	0.132	26.2
10	0.071	0.067	10.6
5	0.034	0.030	4.8
2.5	0.019	0.015	2.4
1	0.013	0.009	1.1
dd H2O Blank	0.004	0.000	0.0

* based on the SRP coefficient derived from the SRP calibration curve on following page

Figure 1- Regression Output

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999725572
R Square	0.999451218
Adjusted R Square	0.999341462
Standard Error	0.766073921
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	5344.085654	5344.086	9106.092	2.40E-09
Residual	5	2.93434626	0.586869		
Total	6	5347.02			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-0.252354078	0.382513977	-0.65973	0.538602	-1.235635952	0.730927796	-1.235635952	0.730927796
X Variable 1	**165.4771839**	1.734091839	95.42585	2.40E-09	161.0195662	169.9348016	161.0195662	169.9348016

A.3.- Total Phosphorus SOP (2005)

Reference Source: Standard Methods 20th Addition- 4500 P.E.
Sample Requirements: Acid preserved, whole water sample

Note: use extreme caution when handling the phosphorus analytical glassware. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described in step three before proceeding any further.

1) Remove a maximum of 29 total phosphorus samples from the freezer and let them thaw. In the morning, make up the following reagents as described in the following section A.1.2-"Mixing other reagents" and in Steps 2 of the A.2.-"Soluble Reactive Phosphorus SOP (2005)":

5 N H_2SO_4
11N H_2SO_4
10N NaOH

2) If the stock TP standard working solutions have expired (see Section 6) k)) then acid wash:

7	1 L Volumetric Flasks
1 ea	2 ml, 4 ml, 10 ml, 40 ml and 50 ml volumetric pipettes
1	glass Petri dish
1	magnetic stirrer

3) Acid wash: (Place in the 30% HCl acid bath for 10 minutes):

84	125 ml Erlenmeyer flasks
1ea	50ml, 100 ml and 250 ml graduated cylinder TD
1	plastic 0.5 g measuring scoop (for ammonium peroxydisulfate)
1	1L volumetric flask
1ea	100 ml, 200 ml and 400 ml beakers
1	10 ml graduated glass pipette

4) Transfer all pertinent information from the total phosphorus sample bottle to the total phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).

5) Preparation of Total Phosphorus stock standard (this step is not required on a daily basis):

A stock phosphorus solution should be prepared monthly using the following procedure:

a) In an acid washed petri-dish pour out approximately 5 grams of anhydrous KH_2PO_4 and place in the oven (Fisher Scientific Model 825F), set at 104°C for one hour.
b) After one hour place the petri dish into a desiccator and let the dish/reagent cool to room temperature. Discard any remaining dehydrated KH_2PO_4 after 5 days.
c) Fill a pre-designated (labeled 50 mg/L TP Standard) 1000ml volumetric flask halfway with DDI H_2O.
d) Add four milliliters of concentrated (36N) sulfuric acid to the solution using a 4ml volumetric pipette and swirl the flask.
e) Carefully weight out 219.5mg KH_2PO_4 on the analytical balance and add the reagent to the pre-labeled volumetric flask. Make sure all reagent is dispensed into the flask. Should some reagent spill onto the counter or onto the outside of the

volumetric flask, discard the reagent, rinse the flask and repeat steps c through f. Note: the anhydrous KH_2PO_4 should be placed in the desiccator between weighings to avoid hydration.

f) Swirl the 1000ml flask for about 20 seconds to dissolve the KH_2PO_4.

g) Bring the 1000ml volumetric flask with DDI H_2O dispensed from a Nalgene squirt bottle.

h) Add an acid washed magnetic stirring bar and place the 1000ml volumetric flask onto a magnetic stirrer set at setting #3. Allow the sample to stir for 30 minutes to assure the reagent reaches a homogeneous state.

i) Label the flask with the current date and place the stock solution into the refrigerator. Any unused portion of this stock reagent should be discarded after 90 days and a new stock phosphorus solution should be mixed at that time.

6) Preparation of working total phosphorus standards and total phosphorus blank.

Note: This step is also not required on a daily basis. However, all total phosphorus working standards and the DDI H_2O blank should be prepared on the same day. Dilute the 50 mg/L stock phosphorus standard, from step 4 above, to a 1 mg/L stock phosphorus solution that will be used to obtain the working standard concentrations described in Table 5.

a) Add 500ml DDI H2O to a pre-labeled (1L) acid washed flask.

b) Draw up 50ml stock phosphorus standard (50 mg/L) into an acid washed 50ml volumetric pipette and dispense the solution in a waste bucket.

c) Draw up a second 50 ml stock phosphorus standard (50 mg/L) into the 50ml volumetric pipette and dispense the solution into the 1L volumetric flask.

d) Bring the 1L (1 mg/L P standard) volumetric flask to volume with DDI H_2O

e) Invert the 1L (1 mg/L P standard) volumetric flask 20 times to obtain a homogeneous mixture.

Table 4. Total Phosphorus working standards.

Standard Solution (*u* g P /L) [1]	1 mg/L Stock Solution (ml) [2]	Erlenmyer Flask (ID) [3]
200	200.0	1
40	40.0	2
20	20.0	3, 13, 23, 33 and 41
10	10.0	4
2	2.0	5
(blank) 0	0.0	6 & 42

[1] Standards used to derive calibration curves (prepared within 28 days of use and stored @ 4°C between uses).
[2] volume of secondary phosphorus stock solution added to the respective 1000ml volumetric flasks.
[3] Erlenmeyer Flasks into which the respective standards and blanks are placed.

f) Six 1000ml volumetric flasks, labeled with the respective standard concentrations: 200 µg/L, 40 µg/L, 20 µg/L, 10 µg/L, 2 μg/L and 0 µg/L (DDI H_2O blank), should be half filled with DDI H_2O.

g) Four milliliters of concentrated H_2SO_4 (36N) should be pipetted, with a class-A volumetric pipette, into each of the six volumetric flasks.

h) Each sample should be swirled to mix the solutions in the respective volumetric flasks.

i) The working standards are then prepared by adding a fixed volume of 1 mg/L stock standard, using class-A volumetric pipettes or a class-A graduated cylinder, into the respective volumetric flasks (Table 4).

j) All volumetric flasks are brought to volume with DDI H_2O, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved.

k) The working standards (0 – 200 µg/L) should be refrigerated between uses and discarded after 28 days. The 50 mg/L stock solution should be refrigerated between uses and discarded after 90 days. The 1mg/L stock standard should be prepared no more than 2 hours prior to the preparation of the working standards. After the working standards are poured, the remaining 1mg/L stock standard should be discarded into a waste bucket, the flask should be acid washed and subsequently rinsed three times with DDI H_2O.

7) Prepare sample flasks as follows:

a) Arrange the 125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 42B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples. Invert each sample ten times and rinse the 50 ml graduated cylinder with the sample. Measure 50 mls of sample and pour into the replicate Erlenmeyer Flasks: A & B (50 mls into each flask). Make sure you mix the water sample ten times before pouring out each replicate stream sample (you often have inorganic particulate debris that rapidly settle out). Between replicate samples (i.e. 1B and 2A) rinse out the graduated cylinder with 30% HCl and then with DDI H_2O. Note: Erlenmeyer flasks 12A and 12B will subsequently be spiked with phosphate standard (as described below) and should contain the same lakewater as was poured into sample 11. Likewise, flasks 32 will subsequently be spiked with phosphate standard and should contain the same lakewater sample as was poured into flasks 31.

b) Pour out the blank as follows: Rinse out the graduated cylinder with 30% HCl and then with DDI H_2O three times. Invert the 1000 ml volumetric flask containing the acidified DDI H_2O blank ten times. Rinse the 50 ml graduated cylinder with approximately 10 ml of your (acidified) DDI H_2O blank. Measure 50 mls of your acidified DDI H_2O blank and pour the blank into the replicate Erlenmeyer flasks (50 mls into each flask).

c) Pour out the Standards as follows: Rinse out the graduated cylinder with 30% HCl and then with DDI H_2O three times. Invert the 1000 ml volumetric flask containing the first phosphorus standard (200 µg/L) ten times. Rinse the 50 ml graduated cylinder with approximately 10 ml phosphorus standard and than measure 50 ml of sample and place into the replicate Erlenmeyer flasks (50 mls into each flask).

Repeat step c for each of the remaining TP standards (40.0 µg/L – 2.0 µg/L). *Note: Your standard curve will be generated daily and will consist of five standards that range from 2.0 - 200.0 ppb.*

8) Spike flasks 12 and 32 as follows:

a) Pour out approximately 30 ml of the 50 mg/L stock Phosphorus standard into an acid washed 100 ml glass beaker.
b) Draw up 50 *u*L of stock standard using a fixed volume micropipetter (50 *u*L MLA Systems D-Tipper, MLA cat # 1054C) and dispense the solution into a waste bucket
c) Draw up 50 *u*L of stock standard using the fixed volume micropipetter and dispense the solution into flask 12A. This phosphorus addition constitutes a 50.0 µg/L phosphorus spike.
d) Repeat step c for flasks 12B, 32A and 32B.

9) Add 1 ml of 11N H_2SO_4 to each Erlenmeyer flask with the Labsystems adjustable pipetter (FS cat # 21-377-109; note: make sure the pipetter is set to dispense 1 milliliter of acid) and then add 1 level scoop (0.5 g plastic scoop HACH cat # 492-00) of ammonium peroxydisulfate into each flask. Cap each flask with a #6 glass stopper (FS Cat # 10-042A) and swirl each flask to assure the reagents are well mixed.

10) Place flasks into the Gettinge Novus I autoclave, adjust the setting to liquids, make sure the temperature is set to 123^{o} C and set the timer to 30 minutes as indicated below.

a) Select "Sterilize Temperature" under the Cycle Values setting and set it to 123.0^{o}C by pressing either the "up arrow" or the "down arrow".
b) Select "Sterilize Time" under the Cycle Values setting and set it to 30 minutes by pressing either the "up arrow" or the "down arrow".
c) Select "Liquids" under the Cycle Select setting. **(It is imperative the autoclave switch is set to liquids, otherwise your samples will vaporize as the autoclave heats up and the samples will be lost).**
d) Once you are sure the settings have been properly adjusted press the "start" button. Stay in the room until the autoclave heats up to 123.0^{o}C to assure the samples are digesting.

11) After 1 to 1.5 hours remove the TP samples from the autoclave using the red thermally insulated gloves.

12) Turn on the Milton Roy Spectronic 1001^{+} at this time. The Milton Roy Spectronic 1001^{+} should be on at least 30 minutes prior to running samples to assure stable readings.

13) As the samples near room temperature mix the following reagents:

Ammonium Molybdate	8.0 grams per 200 milliliters DDI H_2O
Ascorbic Acid	5.28 grams per 300 milliliters DDI H_2O
Potassium Antimonyl tartrate	0.28 grams per 100 milliliters DDI H_2O

14) Once the phosphorus samples cool to room temperature (25^{o}C) remove the caps and add one drop phenolphthalein indicator to each flask. Neutralize each sample to a faint pink color by dispensing 1.4 ml 10N NaOH from the Labsystems pipetter into each flask. *Note: make sure the volume is set to 1.4 ml before beginning*. Following the addition of NaOH to all samples,

swirl each flask individually; the pink color should disappear at this point. If the pink color persists, however, consult the laboratory manager.

15) Mix the combined reagent: The combined reagent should be mixed in a 1000 ml (acid washed) volumetric flask by measuring the volume of reagents in a graduated cylinder and adding the reagents in the following order (note: the reagents must be added in this order for the proper molecule to form and the graduated cylinder should be rinsed with DDI H_2O between the addition of each reagent); mixing the volumetric flask as each reagent is added:

500 mls 5N H_2SO_4
50 mls Antimony Potassium Tartrate
150 mls Ammonium Molybdate
300 mls Ascorbic Acid

Note: the mixed reagent is very unstable and should be made immediately prior to adding to the sample flasks.

16) Add the mixed reagent using the yellow and black pipetter (FS Cat# 13-681-25), rinsing the pipette by pipetting a sample of mixed reagent from the beaker and discarding it. After rinsing the pipette tip, add 8 mls of mixed reagent to each successive flask. As the mixed reagent is added a molecular complex (molybdenum blue) will form in the sample. The concentration of the molybdenum blue complex is proportional to the phosphorus concentration in the sample. While differences in color (low phosphorus concentrations) are not visible to the unaided eye, high phosphorus concentrations become various shades of blue; the bluer the sample the greater the phosphorus concentration. See Procedures in Section A.1.5 and A1.3.b. for proper use of the spectrophotometer.

17) Begin sample analysis 30 minutes after adding the mixed reagent to the first sample. Record the absorbencies at 660 and 880nm on the total phosphorus data sheet. The spectrophotometer should be blanked with DDI H_2O and blanks should also be run after every 10 flasks and should always be run after the final phosphorus sample has been run. Record the blank absorbencies (660 and 880nm) and the DDI H_2O blank results on the datasheets.

18) All phosphorus samples should be poured into a white (one-gallon) paint bucket and neutralized with baking soda prior to disposal.

19) Glassware cleanup - Immediately rinse out the flasks and other glassware three times with DDI H_2O after the run and place the glassware in the drying rack on the Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let sit for one hour, otherwise, fill the flasks with DDI H_2O and place out of the way until room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DDI H_2O and place the glassware upside-down in the drying rack.

20) <u>Calculating the Total Phosphorus (TP) Coefficient from the calibration curve data:</u>

After each analytical run enter the five initial TP standards (samples 1-5) and the corresponding 880 nm absorption into an Excel spreadsheet as depicted in the first two columns of Table 6. Subtract the DDI H_2O blank 880nm absorption value (sample 6) from each of the 880nm standard absorption values as displayed in column 3 of Table 6. Regress the standards (dependent variable) against the corrected 880nm absorption values (independent variable) to generate a statistical output as displayed below. You will now

multiply your corrected 880nm absorption values by the X variable (TP coefficient) to calculate your TP concentrations (Table 5). As you can see, the prepared TP standards compare well with the calculated TP values. You will now use your TP coefficient and the corrected 880nm absorption values of your samples (unknowns) to determine the remaining TP concentrations. You should record your TP coefficient, your DDI H_2O blank (corrective factor) and your r^2 value on the back of your Total Phosphorus datasheet.

Table 5: Total Phosphorus Calibration curve

TP Standard (ppb)	880nm abs	880 nm abs (corrected for blank)	* Calculated TP (ppb)
200	1.008	1.005	198.5
40	0.213	0.210	40.9
20	0.110	0.107	20.6
10	0.059	0.056	10.4
2	0.016	0.013	1.9
dd H2O Blank	0.003	0.000	0.0

* based on the TP coefficient derived from the TP calibration curve below

Figure 2 Regression Output

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.999725572
R Square	0.999451218
Adjusted R Square	0.999341462
Standard Error	0.766073921
Observations	6

ANOVA

	df	SS	MS	F	Significance F
Regression	1	5344.085654	5344.086	9106.092	2.40E-09
Residual	4	2.93434626	0.586869		
Total	5	5347.02			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Up
Intercept	-0.252354078	0.382513977	-0.65973	0.538602	-1.235635952	0.730927796	-1.235635952	0
X Variable 1	**198.4771839**	1.734091839	95.42585	2.40E-09	161.0195662	169.9348016	161.0195662	1

Written by Bob Craycraft and Jeff Schloss
Last Updated on 02/23/05
c:/CFBdocuments/SOPs/SOPL2005TP.doc

A.4.- Total Nitrogen SOP (2005)

(via Second Derivative Spectroscopy for Nitrate N)

Reference: Standard Methods 4500NO_3.C

Additional References:

Bachmann, R. and D.E. Canfield. 1996. Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. Hydrobiologia. 323:1-8.

Crumpton, W.G. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnol. Oceanogr. 37(4): 907-913

1) Remove up to 22 samples from the freezer and allow to thaw. Acid Wash (30% HCL): 57-Culture Tubes and screw caps, 1-500 ml beaker, 1- 50 ml beaker

Note: See also sections for nitrogen stock solutions (A through C) if they have expired (shelf-life listed in Section C).

A-Preparation of Nitrogen Stock :

A stock potassium nitrate (KNO_3) solution should be prepared every three months and will be used to derive our working nitrogen standards as described below:

1) Place approximately 5g KNO3 in the oven at 105^oC for 24 hours.
2) After 24 hours place the dehydrated KNO_3 in a desiccator and allow the reagent to cool to room temperature.
3) Fill a pre-labeled (1000mg/L Nitrogen Stock), acid washed, 1L volumetric flask with approximately 500ml DDI H_2O.
4) Carefully measure out 0.7218g KNO_3 and pour all the reagent into the 1L volumetric flask. *Note: should any reagent spill onto the benchtop or onto the outside of the flask, you should rinse the volumetric flask five times with DDI H_2O and repeat the steps 3 and 4. Always return the dehydrated KNO_3 to the desiccator after pouring out the reagent.*
5) Add 4ml 36N H_2SO_4, dispensed via an acid washed volumetric pipette, to the 1L volumetric flask.
6) Swirl the flask to dissolve the KNO_3 crystals.
7) Bring the flask to volume by slowly adding DDI H_2O dispensed via a Nalgene squirt bottle.
8) Parafilm the 1L flask and invert twenty times or until all the KNO_3 has dissolved.
9) Store the stock 1000 mg/L nitrogen standard in the refrigerator until needed. This solution should be discarded after 90 days.

B-Intermediate Nitrogen Stock:

An intermediate Nitrogen stock solution (10 mg/L Nitrogen Stock) should be prepared immediately prior to preparing the working nitrogen standards as described below:

1) Fill a pre-labeled (10 mg/L Nitrogen Stock), acid washed, 1L volumetric flask with approximately 500ml DDI H_2O.
2) Draw up 10ml of 1000 mg/L Nitrogen Stock in an acid washed 10ml volumetric pipette and discard the solution in a waste bucket.
3) Draw up 10ml of 1000 mg/L Nitrogen Stock) in the 10ml volumetric pipette and dispense the solution into the 1L volumetric flask (10 mg/L Intermediate Nitrogen Standard).
4) Swirl the volumetric flask to obtain a homogeneous mixture.
5) Bring the 1L flask to volume by slowly dispensing DDI H_2O from a Nalgene Squirt bottle.
6) Parafilm the 1L flask and invert 20 times to obtain a homogeneous mixture.

C-Preparation of Working Standards and DDI H_2O blank:

The Intermediate Nitrogen Stock Solution should be diluted as outlined below and as summarized in Table 6 to obtain your working standards:

1) Seven 1000 ml volumetric flasks, labeled with the respective standard concentrations: 3000 µg/L, 2000 µg/L, 1000 µg/L, 500 µg/L, 250 µg/L, 100 µg/L and 0.0 µg/L (DDI H_2O blank), should be half filled with DDI H_2O.
2) Four milliliters of concentrated H_2SO_4 (36N) should be pipetted, with a class-A volumetric pipette, into each of the seven volumetric flasks.
3) Each sample should be swirled to mix the solutions in the respective volumetric flasks.
4) The working standards are then prepared by measuring a fixed volume of Intermediate Nitrogen Standard (10 mg/L) into the respective volumetric flasks using either a class-A volumetric pipette or a class-A graduated cylinder as documented in Table 6.
5) All volumetric flasks are brought to volume with DDI H_2O, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions are achieved.

The working standards (0 – 3000 µg/L) should be refrigerated between uses and discarded after 28 days. The 1000 mg/L stock solution should be refrigerated between uses and discarded after 90 days. The 10 mg/L intermediate nitrogen solution should be prepared no more than 2 hours prior to the preparation of the working standards. After the working standards are poured, the remaining 10 mg/L intermediate nitrogen standard should be discarded into a waste bucket, the volumetric flask should be acid washed and subsequently rinsed three times with DDI H_2O.

Table 6. Nitrogen working standards.

Working Standard Solution (µg/L) [1]	Intermediate Nitrogen Stock Solution (ml) [2]	Culture Tube (ID) [3]
3000	300.0	2
2000	200.0	3
1000	100.0	4, 16, 27, 42 & 56
500	50.0	5
250	25.0	6
100	10.0	7
(blank) 0	0.0	1 & 57

[1] Standards used to derive calibration curves (prepared within 28 days of use).
[2] volume of intermediate nitrogen stock added to a 1L volumetric flask and brought to volume with dd H_2O water to derive the working standards presented in the leftmost column above. The 500-3000 µg/L standards are prepared by measuring the Intermediate Nitrogen Stock in a Graduated Cylinder while the 100 and 250 µg/L standard are prepared by measuring the Intermediate Nitrogen Stock in volumetric pipettes.
[3] Culture tube into which the respective standards and blanks are placed.

D-Preparing samples, standards and blanks for analysis:

1) Record the appropriate information (i.e. lake, site, date, depth) on the Total Nitrogen datasheet (Appendix C) and proceed as follows:
   a) Invert the first sample (DDI H_2O blank) 10 times.

b) Measure out 30 milliliters of sample into an acid washed 50 ml class-A graduated cylinder.
c) Pour the sample into the properly labeled test tube (Pyrex 25x150 mm culture tube w/screw cap, cat #9826-25) and place the cap loosely on the sample.
d) Wash the graduated cylinder with 30% HCl between samples and rinse the graduated cylinder three times with DDI H_2O.
e) Repeat steps a through d with successive samples. Note: samples 12 through 15 and samples 38 through 41 are laboratory replicates and spikes and should contain water poured from the same samples.

2) Samples 14, 15, 40 and 41 should be spiked with 1000 mg/L Nitrogen stock standard using the following procedure:
a) Pour approximately 20 ml of 1000 mg/L nitrogen stock standard into an acid washed 50 ml beaker.
b) Draw up 50 *u*L of stock standard using a fixed volume micropipetter (50 *u*L MLA Systems D-Tipper, MLA cat # 1054C) and dispense the solution into a waste bucket
c) Draw up 50 *u*L of stock standard using the fixed volume micropipetter and dispense the solution into sample 14. Loosely cap the test tube after adding the spike and gently swirl the sample. This nitrogen addition constitutes a 1664 µg/L nitrogen spike.
d) Repeat step c for samples 15, 40 and 41.

3) Add 4.5 milliliters of potassium persulfate (oxidizing agent preparation described below) to each test tube using the following procedure:
a) Pour the Alkaline Potassium Persulfate oxidizing agent into an acid washed 500 ml beaker.
b) Draw up 4.5 ml Alkaline Potassium Persulfate oxidizing agent using a 5.0 ml adjustable Thermo Labsystems Finpipette (cat # 40270-280) and discard the solution into a waste bucket.
c) Draw up 4.5 ml Alkaline Potassium Persulfate oxidizing agent and dispense the solution into test tube #1 (DDI H2O blank). Loosely cap the test tube.
d) Dispense 4.5 ml Alkaline Potassium Persulfate into each of your remaining test tubes (#2 through #57) as described in step c above.
e) Tighten each test tube cap after all samples have received an aliquot of oxidizing agent. Gently swirl each sample.

4) Place the samples and the test tube rack (the rack is autoclavable) into a Nalgene Polypropylene autoclavable bin and autoclave the samples for 30 minutes at 123^{o}C. Note: be sure the autoclave is set to <u>liquids</u> before initiating the digestion as described in the autoclave use directions.

<u>E-Oxidizing Agent Preparation:</u>

Dissolve 6.0g Potassium Persulfate in 100mls 2.0N NaOH = Alkaline Potassium Persulfate solution. (2.0N NaOH solution will neutralize the acid preservative added and maintain a basic environment for the digestion) – All lakewater samples, standards and blanks were preserved with 1 milliliter H_2SO_4/250 milliliters of lakewater.

<u>F-Post-Autoclave treatment:</u>

1) Place test tube-rack into an ice bath and cool the samples to room temperature before proceeding to step 2. Note: it is imperative that the samples are cooled before the addition of acid – otherwise a volatile reaction will ensue.
2) Turn on the Cary 50 spectrophotometer if you have not already done so.
3) Add 0.6 milliliters concentrated H_2SO_4 to each test tube using a Wheaton Model 851350 (0.5 – 5 milliliter) adjustable macropipetter.
4) Cap each test tube and invert each test tube three times.
5) Analyze the samples on the Cary 50 scanning spectrophotometer using the procedure outlined in "Spectrophotometric Analysis on the Cary 50 spectrophotometer".

G-Spectrophotometric Analysis on the Cary 50 spectrophotometer

1) Select the icon "Shortcut to Nitrogen.MSW" from the desktop to initiate the Total Nitrogen analysis program. If the icon "Shortcut to Nitrogen.MSW" does not appear on the desktop consult the laboratory manager before proceeding to step two (the total nitrogen analytical settings are included below in the event the "Nitrogen.MSW" file has been lost or modified).
2) Select the Total Nitrogen Cuvette (16 milliliter, 5 cm path length, cylindrical quartz cuvette, FS cat # 14-385-930E) and rinse with DDI H_2O three times.
3) Fill the Total Nitrogen cuvette with DDI H_2O, gently tap the cuvette to dislodge any air bubbles, wipe the cuvette faces with a Kimwipe and inspect the cuvette to assure no smears or particles will impede the light path. Insert the sample into the spectrophotometer cell holder and close the lid. *Note: the cuvette should be centered in the cell holder and care should be taken to place the cuvette in the same position with each subsequent water sample.*
4) Select "baseline" from the list of options indicated on the computer monitor (this will set all absorbency values between 250nm and 200nm to zero).
5) Empty the cuvette into a waste bucket and fill the cuvette with the contents of test tube #1. Repeat step #3 to assure the sample is ready for analysis (tap the cuvette to dislodge any air bubbles, wipe the cuvette faces with a Kimwipe and inspect the cuvette to assure no smears or particles will impede the light path and result in errors. Insert the sample into the spectrophotometer cell holder and close the lid).
6) Press "Start" You will then be prompted for a file name – proceed as follows:
    a) Create a new folder in the CaryWinUV sub-directory by clicking on the create folder icon. Name the folder TN"today's date" (e.g. TN021402 which stands for Total Nitrogen analysis that occurred on February 14, 2002).
    b) Double click the folder you just created (e.g. TN021402) to set this folder as the location where your files will be stored.
    c) You are now prompted to enter a file name. Use the format: Site ID, replicate # (e.g. for the first sample on the datasheet, DDI H_2O blank, you would enter "DDI H2O blank replicate 1". For a sample collected from Swains Lake, Site A on June 2, 2002, collected at a depth of 5.0 meters you would enter "Swains Lake Site A depth 5 060202". Use the format of month, day, year as indicated in the example. This program will not accept non-alphanumeric characters such as "/", "-" or ".".). If you have a depth that includes a decimal place (e.g. 5.5 meters) enter the depth as 55.
    d) Once you have entered the file name press the "return" key.
    e) You are now prompted for a sample name. Use the same format as used for the file name (e.g. Swains Lake Site A depth 5 060202).
    f) Once you have entered the sample name press the "return" key.
    g) You can now prompted to select "OK" or "cancel". Press "OK" to begin the analysis. The spectrophotometer will scan and record the absorption data between 200 and 250 nm and will cycle through three times – this takes approximately six minutes. At the end of the third cycle all data will automatically be saved to a computer file in the folder (e.g. TN021402) that you created previously.
7) Remove the cuvette from the spectrophotometer chamber, pour the cuvette contents into the waste bucket and rinse three times with DDI H_2O. Fill the cuvette with the next sample and repeat step 3 to assure the cuvette has been properly treated/cleaned.
8) Repeat Step 6 typing in the appropriate information for your current sample.
9) Repeat Steps 6 and 7 until all samples have been analyzed.
10) Once all samples have been analyzed we can proceed to the total nitrogen second derivative calculations step described on the next page.

H-Total Nitrogen analytical Settings

Select "setup"

X mode: the scan range should be set from "200" nm to "250" nm.

Cycle count: the cycle count should be set to "3"

Scan Controls - the scan controls should be set to:

1) Average time(s) = "0.5" seconds
2) Data Interval (nm) = "0.25" nm
3) Scan Rate (nm/min) = "30.0" nm/minute

I-Total Nitrogen second derivative calculations

1) a) Select the calculator icon from the menu bar (right hand side)
   b) Select "selected graph" from the display options
   c) Select "selected trace"
   d) Set the operation to "Deriv2"
   e) Set the filter size to "17"
   f) Set the interval to "0.5"
   g) Press the "Apply" button
2) a) Select trace preferences icon from the menu bar (left hand side)
   b) Select the appropriate file by selecting the "color cell" – object in the cell will become red and indicates it is selected
   c) Press "OK"
3) a) Select the calculator icon again (notice the equation that you generated in step one is displayed)
   b) Select the "=" sign
   c) At the Y label prompt select "2nd derivative" from the drop down menu.
   d) Press "OK" (you have now generated a new "Deriv 2 file" that can be viewed by selecting the traced preferences icon.
6) Repeat steps 2 and 3 until you have generated second derivative files for all your data.
7) Once you have calculated second derivatives for all of your data select "save data as" from the file menu. Name the file "second derivatives" and press the "save" button. All of your second derivative data is now saved and can be archived for further reference.
8) Record the 226.5nm second derivative peaks on the total nitrogen datasheet for each sample analyzed. Note: 226.5nm should represent the maximum second derivative peak for each sample processed. If the peak is located at another wavelength consult the laboratory manager before recording any data.

J-Total Nitrogen Daily Calibration Curve

After each analytical run the six initial TN standards (samples 2-7) and the 2nd derivative values should be entered into an Excel spreadsheet as depicted in the first two columns of Table 5. Subtract the DDI H_2O blank 2nd derivative value (sample 1) from each of the TN Standard 2nd derivative values displayed in the "Corrected 2nd der" column. Regress the standards (dependent variable) against the corrected 2nd derivative values (independent variable) to generate a statistical output as displayed below. You will now multiply your corrected 2nd derivative values by the X variable (TN coefficient) to calculate your TN concentrations (Table 7). As you can see, the prepared TN standards compare well with the calculated TN values. You will now use your TN coefficient and the corrected 2nd derivative values of your samples (unknowns) to determine the remaining TN concentrations. You should record your TN coefficient, your 2nd derivative DDI H_2O blank (corrective factor) and your r^2 value on the back of your Total Nitrogen datasheet.

Table 7: Total Nitrogen Calibration curve

Standard	2nd der average	Corrected 2nd der	calculated TN (ug/L) *
dd H_2O Blank	0.000084	0.000000	0
2000	0.004481	0.004397	1995
1000	0.002285	0.002201	999
500	0.001224	0.001140	517
250	0.000650	0.000566	257
125	0.000360	0.000276	125

* based on the Total Nitrogen coefficient derived from the Total Nitrogen calibration curve below.

Figure 3 Regression Output

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999921079
R Square	0.999842164
Adjusted R Square	0.749842164
Standard Error	9.578222215
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2324633.031	2324633.031	25338.71504	5.46679E-07
Residual	4	366.9693632	91.7423408		
Total	5	2325000			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	0	#N/A	#N/A	#N/A
X Variable 1	453819.5733	1883.197571	240.9835167	1.7789E-09

Written by Bob Craycraftand Jeff Schloss
Last Updated on 02/23/05
c:/CFBdocuments/SOPs/SOPL2005TN.doc

A.5.-Solids/Percent Organic Matter SOP (2005) (Sediment or sludge sample)

Reference: Standard Methods 20th Addition Method 2540.G.

Additional Reference:
USGS 1975 Methods for the Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the United States Geological Survey, Book 5, Chapter A1 Edited by Marvin J. Fishman and Linda C. Friedman
I-5753- Solids, Volatile-on-ignition, Total-in-bottom-material, Gravimetric

A portion of well-mixed sediment sample is dried at up to 105^{o}C. A portion of that dry sample is carefully weighed and then ignited at 550^{o}C. The loss of weight on ignition represents the amount of volatile solids in the sample. The volatile solids of a sample roughly approximate the organic matter content.
Analyze at least 10% of all samples in duplicate.

1-Work-up (sediment drying)

a) Preheat the oven (Fisher Scientific (FS) Model 825F) to 104^{o}C +/- 1^{o}C.
b) Transfer the pertinent information (i.e. lake, site, collection date, sample depth, etc.) from the sampling bottle to the Percent Organic Matter Datasheet.
c) Place the evaporating dishes (Pyrex no. 3180) into an acid bath (30% HCl) for 10 minutes and then rinse three times with DDI H_2O.
d) Place evaporating dishes into the oven for one hour. Heat dish at 103°C to 105°C for 1 h in an oven.
e) Cool in the desiccator, weigh, record weights on data sheet, and store in the desiccator until ready for use.
f) Arrange the numbered evaporating dishes in sequential order.
g) Pour 25-50 gms of the appropriate benthic sample into the corresponding evaporating dish (as indicated on the datasheet).
h) Place the evaporating dishes into the oven and let the samples dry for 24 hours.
i) Remove the evaporating dishes, place in a large glass dessicator and allow the samples to cool to room temperature. Note: if the dessicant is not blue but pink consult the laboratory manager to proceed with dessicant replacement and to assure the dessicators have an airtight seal.
j) Repeat drying (1 h), cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less. Record the final weight on the datasheet.

2-Pre-Ash Weight

a) Preheat the muffle furnace.
b) Place up to 9 quartz ashing dishes (FS Cat # 08-072c) into an acid bath (30% HCl) for 10 minutes and then rinse three times with DDI H_2O.
c) <u>Put on the orange thermally insulated gloves labeled "Furnace"</u>. These are the only gloves that are resistant to the high furnace temperature and it is imperative that you put them on before proceeding.

d) Place the quartz ashing dishes into the Thermolyne model 48000 muffle furnace using the crucible tongs (FS Cat # 15-210) and orange protective gloves.
e) Ignite the acid washed ashing dishes at 550°C for 1 h.
f) Place the quartz ashing dishes into an empty small glass dessicator using the crucible tongs and orange protective gloves. Make sure the desiccant is blue, not pink- otherwise have the lab manager replace the desiccant.
g) Tare the Denver Instruments A-250 analytical balance.
h) Place the first quartz ashing dish on the balance, close the door and let the weight stabilize. Record the weight on the Percent Organic Matter datasheet. Repeat for all other ashing dishes.
i) Remove your first benthic sample from the large glass dessicator and pulverize it (in the evaporation dish) with a pestle until you have obtained a "homogeneous mixture". Make sure you immediately replace the dessicator lid between samples.
j) Carefully pour between 3 and 5 grams of benthic matrix into the Quartz ashing dish. Note: pour this out carefully to minimize the amount of airborne particles (fine organic and inorganic debris). You may want to save the remainder for Sediment Total Phosphorous or Total Nitrogen digestion and analysis. If so, cover the evaporation dish with a square of aluminum foil shiny side up and replace in the glass dessicator. Start digestion as soon as possible, optimally within 24 hours.
k) Measure the weight of the ashing dish + the benthic matrix you just added on the analytical balance. Record the total weight of onto the benthic datasheet.
l) Place the filled Quartz ashing dish into an empty dessicator. Make sure you place the ashing dish in the appropriately numbered slot (i.e. sample one should be placed into slot one). This placement is critical since the ashing dishes are not numbered.
m) Carefully wipe off the pestle with a kimwipe. Note: the pestle should not have any moisture on it. If there is any doubt place the pestle into the FS Model 825F oven for 10 minutes and subsequently remove the pestle while wearing the orange, thermally insulated, laboratory gloves.
n) Repeat the Pre-Ash Steps i) through m) until you have poured out up to 9 samples to be ashed.

3-Ashing Procedure

a) **Put on the orange thermally insulated gloves labeled "Furnace".** ***These are the only gloves that are resistant to the high furnace temperature and it is imperative that you put them on before proceeding.***
b) Place the Quartz ashing dishes into the Thermolyne model 48000 muffle furnace using the crucible tongs. Arrange the first three samples on the top shelf from left to right, samples four through six on the bottom shelf in back from left to right, and samples:

Table 8 Position of ashing trays in muffle furnace

	Left	Center	Right
Top Shelf	Sample #1	Sample #2	Sample #3
Bottom Shelf Back	Sample #4	Sample #5	Sample #6
Bottom Shelf Front	Sample #7	Sample #8	Sample #9

c) If the furnace has reached temperature (550^{o}C) skip to e).

d) Turn on the Muffle Furnace and let it warm up to the pre-set temperature of 550^{o}C.
e) Leave the samples in the oven for one hour from the time the temperature reaches 550^{o}C. Note: you are incinerating the organic matter in the samples and it will smell like something is burning for a short period – longer if there is appreciable organic matter in the sample. Put a note on both laboratory doors (in big bold letters) that indicates we are ashing samples and there is no fire.
f) You should turn the muffle furnace off after one hour at 550^{o}C.
g) Place a glass dessicator on one of the Rubbermaid carts and wheel it over to the Muffle Furnace.
h) Remove the lid to the dessicator and place it to the side.
i) Using the crucible tongs, carefully pick the ash dishes up one by one and place them in the appropriately numbered slits in the dessicator.
j) Once all samples have been place in the dessicator put on the lid. Note: you should slide the lid to the side every two minutes (for the first 10 minutes) to release the pressure. If you do not release the pressure the samples will cool and will create a vacuum seal that is extremely difficult to "break".
k) After a one-half hour cool down period the ashed samples are ready to be weighed (the change in weight is proportional to the amount of organic matter in the samples).

4-Post-Ashing Weighing Procedure

a) Tare the Denver Instruments A-250 analytical balance.
b) Place the first Quartz ashing dish on the analytical balance, close the door and let the weight stabilize. Record the post-ash weight on the datasheet.
c) Repeat igniting (30 min), cooling, desiccating and weighing steps until the weight change is less than 4% or 50 mg, whichever is less.
d) Remove the Quartz ashing dish from the analytical balance and place it on the laboratory bench for cleaning (all data have been recorded so there is no need to return the sample to the dessicator).
e) Repeat steps "a" through "c" with subsequent samples until all samples have been analyzed and the pertinent data have been recorded.
f) Analyze at least 10 percent of all samples as duplicates.

5-Post Analysis Clean-Up

a) Take both the desiccation dishes and the ashing dishes to the sink and rinse them out with DDI H_2O. If necessary, use a laboratory cleaning brush and Alconox to remove any stubborn sediment from the glass and then rinse liberally with DDI H2O to assure all particulate debris had been washed away.
b) Wipe down the analytical balance with a laboratory sponge to remove any dust that had settled out.
c) Turn off the Denver Instruments A-250 analytical balance.

6-Calculations

a) Percent Total Solids:

% Total Solids = 100 x (A - B) / (C - B)

where:
A = weight of dried residue and evaporating dish, in mg,
B = evaporating dish weight dish in mg,
C = weight of wet sample and evaporating dish, in mg.

b) Percent Volatile and Fixed Solids:

% Volatile Solids = 100 x (D - F) / (D - E)

% Fixed Solids = 100 x (F - E) / (D - E)

where:
D = weight of dried residue and ashing dish, in mg,
E = ashing dish weight, in mg,
F = weight of residue and ashing dish after ignition, in mg.

A.6.-Digestion of Samples for Total Phosphorus and/or Total Nitrogen (Sediment samples)

A.6.1-Microwave Digestion-

Due to the caustic nature of the HCl/HNO3/HFlO digestion media and specialized equipment (Microwave Digester, Teflon Pressurized Digestion Vessels etc.) we utilize the UNH Instrumentation Center (Department of Chemistry) facilities, equipment (CEM MDS 2000 System) and staff. Make arrangements in advance to bring samples to the lab. Digestions can be run according to any of the appropriate methods such as EPA 3051, 3051A, 3050B, 3015, 3015A, etc.

1- Weigh out proper sample size for technique on tared plastic acid washed disposable weighting dish if not already in evaporation dish or ashing dish. Use dried (105^{o}C) ashed (see SOP A.5. for either), or freeze dried sample depending on request. Carefully transport samples to instrumentation center using a dessicator. Make sure you also bring the proper labeled containers for them to add the digest into when the procedure is complete.

2- When sample are digested run the appropriate Total P or Total N test or both according to the CFB lab SOPs. Generally you will dilute 1 ml of the sample into 49 ml of water to bring the samples down to the proper range and negate any turbidity interference. Do all dilutions under the laboratory hood as the digests used are extremely reactive. Dispose of the samples properly as directed by the lab manager.

A.6.2-Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus- (Modified for sediment digestion)

References: Standard Method ("new" online and upcoming 21st edition) 4500 P J. and Simultaneous Determination of Total Nitrogen and Total Phosphorus in Freshwater Samples Using Persulfate Digestion. Hosomi, M; Sudo, R International Journal of Environmental Studies IJEVAW Vol. 27, No. 3/4, p 267-275, 1986.

Sample requirements: Non-acidified fresh or frozen original sample dried, freeze dried or dried then ashed.

Through comparison tests between this method, hot acid and microwave digestions we have found that this method is preferable for the determination of both N and P for lake sediments as it does not require HNO3 or other extremely volatile acids that require great care and special air exhaust areas.

This simultaneous digestion method gives not only a high recovery and reproducibility over a wide range of various nitrogen and/or phosphorus compounds of known nitrogen and phosphorus compositions, but also high recoveries of nitrogen and phosphorus from standard reference materials, stream leaf matter, lake sediments, and algal cultures. No significant difference was found between the determinations of T-N and T-P in freshwater sediment samples by this method and those determined

by the standard methods of T-N and T-P, respectively. This method should be useful for the routine analysis of T-N and T-P in freshwater samples containing particulate material and sediment samples.

1- Reagents and Standards:
   a. **Sodium hydroxide, 3N**: Slowly dissolve 120 g low-nitrogen NaOH in 800 ml DDI water in a 1000-ml volumetric flask placed in ice. Cool and dilute to volume.
   b. **Oxidizing reagent**: Dissolve 64 g low-nitrogen (<0.001%N) potassium persulfate, $K_2S_2O_8$, in 500 ml deionized water in a 1L volumetric flask. Use low heat if necessary. Add 80 ml 3N NaOH, that was just prepared from the low-nitrogen sodium hydroxide, and dilute to 1000 ml. Store in a brown glass bottle at room temperature.
   c. **Urea Standard Stock Solution and Standard:** (Organic N control standard) Dissolved 2.1437 g of urea in 1 L of DDI water. 1.0 ml of this solution contains 1 mg of N. Working Calibration Standard Solution: Dilute 2.0 ml of urea stock solution to 100 ml in a volumetric flask with DDI water to create a 20 mg/L organic nitrogen control standard. Cover flask with parafilm and invert 10 times to mix. (20mg/L standard).
   d. **Adenosine Triphosphate Stock Solution and Standard**: (Organic P control standard) Dissolve 0.6514 g adenosine triphosphate in deionized water and dilute to 1000 ml; 1 ml = 0.1 mg P. To prepare a calibration standard, dilute 10.0 ml stock solution to 100 ml in a volumetric flask and bring to volume with DDI water. Cover flask with parafilm and invert 10 times to mix. (10mg/L standard)
   e. **Orthophosphate Control Standard:** Use previously prepared 10-mg/L P stock standard (ie: the 30 ppm phosphate stock standard).
   f. **Nitrate Control Standard:** Prepare a 50 mg/L Nitrate Standard by diluting 5 ml of stock potassium nitrate (KNO3) solution (1000 mg/L N) to 100 ml in a volumetric flask (final concentration 50 mg /L). Cover flask with parafilm and invert 10 times to mix.
   g. In addition to the above reagents mix any required reagents and standards for the total nitrogen and total phosphorus determinations.
2- Use the “Sediment Digestion’ lab data sheets, as well as the specific Sediment TP or TN datasheets
3- Set up the 250 ml flasks in the autoclave bin so that you have one for each sample as well as 6 extra for controls and blanks. Also have a 500 ml flask ready.
4- Add 48 ml of DDI water to all 250 ml flasks except 1A through 3A and 4B. Add 240 ml DDI water to the 500ml flask.
5- Leave flask 1A as the blank.
6- For flask 1B, 2A, 2B, and 3A add 48 ml of the nutrient control standard solutions: Urea, ATP, phosphate and potassium nitrate to each flask respectively. Use a 50 ml graduated cylinder that is acid rinsed and then rinsed with DDI water three times between each standard.
7- Weigh out proper sample size for technique chosen on tared disposable weighting paper. Use dried (105^{o}C) and or ashed (see SOP A.5. for either), or freeze dried sample depending on request. Before and after weighting keep samples in the dessicator.
   a. One sample will be weighed out 3 times for a duplicate sample and a matrix spike. For dried samples or freeze dried samples: Weigh 5.0 +/-0.2 grams of the dry sample #1 into the flasks 3B, 4A and 4B. Write the flask number and weight of sample onto the datasheet.

i. Into flask 4B also add 10 ml of 30 mg/L Stock Phosphate Standard and 10 ml of 50 mg/L Nitrogen Working Standard. Add 28 ml of DDI water.

b. For the remaining dried samples or freeze dried samples: Weigh 5.0 +/-0.2 grams of the dry sample into the flask, write flask number and weight of sample onto the datasheet. Start at flask 5A.

8- Add 10 ml of oxidizing reagent to each flask using the 10.0 ml repeater pipette. Cap each flask with a #6 glass stopper (FS Cat # 10-042A) and swirl each flask to assure the samples are well mixed.

9- Add 50 ml of oxidizing reagent to the 500 ml flask using a graduated cylinder. Cap the flask with a #8 glass stopper and swirl to mix.

10- Place flasks into the Gettinge Novus I autoclave, adjust the setting to liquids, make sure the temperature is set to 123^{o} C and set the timer to 90 minutes as indicated below.

a. a) Select "Sterilize Temperature" under the Cycle Values setting and set it to 123.0^{o}C by pressing either the "up arrow" or the "down arrow".

b. Select "Sterilize Time" under the Cycle Values setting and set it to 60 minutes by pressing either the "up arrow" or the "down arrow".

c. Select "Liquids" under the Cycle Select setting. (It is imperative the autoclave switch is set to liquids, otherwise your samples will vaporize as the autoclave heats up and the samples will be lost).

d. Once you are sure the settings have been properly adjusted press the "start" button. Stay in the room until the autoclave heats up to 123.0^{o}C to assure the samples are digesting.

11- Turn on the appropriate spectrophotometer : Milton Roy 1001+ for TP, Cary 50 for TN.

12- Retrieve samples after 2.0 – 2.5 hours. In the meantime set up the appropriate glassware as indicated in sections 13 I and 13 II below.

13- When samples are sufficiently cooled, add 0.2 ml of 3N NaOH using the fixed volume pipettor to each 250 ml flask. Gently swirl the flasks after addition.

14- Add 2.0 ml of 3N NaOH using a fixed volume Class A 2.0ml volumetric pipette to the 500 ml flask and swirl to mix.

I- **For TP analysis** prepare sample and standard flasks as follows:

a. Prepare enough 125 ml TP Erlenmeyer flasks (Corning # 5100-125) to account for twice the number of flasks you used in the digestion plus 6 more. Arrange the flasks alpha-numerically, 1A through #B (where # = one half of the total number of flasks used), and place sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.

b. Prepare TP standards and blank as follows (for calibration curve):

i. Label 7 clean 500 ml, volumetric flasks with the respective standard concentrations: 2000 µg/L, 1000 µg/L, 500 µg/L, 100 µg/L, 50 µg/L, and 10 µg/L.

ii. Using a 25 ml graduated cylinder add 20 ml of digestion blank solution from the 500 ml flask into each volumetric flask.

Table 9. TP working standards for Sediment TP.

Standard Solution (µg/L) [1]	HACH Stock Solution (ml) [2]	1000 µ/L Solution (ml)[3]	Erlenmyer Flask (ID) [4]
(blank) 0	0.0	0.0	1A
2000	20.0	0.0	1B
1000	10.0	0.0	2A
500	5.0	0.0	2B
100	1.0	0.0	3A
50	0.5	0.0	3B
10	0.0	5.0	4A
5	0.0	2.5	4B

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] volume of stock HACH standard (HACH cat # 1436716) added to a 500 ml volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above.
[3] volume of 1000 µ/L calibration working standard added to a 500ml volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer Flasks into which the respective standards and blanks are placed.

d

Prepare Standard Flasks as Follows: Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/L stock standard, as 30.0 mg/L PO4-3, into the appropriate standard concentrations described in Table 9. For the last two standards you will utilize the 1000 u/L solution you will have just mixed.

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

c. All volumetric flasks are then brought to volume with DDI H2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved. Pour out 50 ml of each of the standards into the appropriate flasks as shown in Table 9.
d. To all TP flasks starting with 5A add 48ml of DDI water.
e. To minimize the impact of turbidity interference from the digested solution, the sediment digestions are diluted 1:25. Draw up 2.0 ml of the blank in digested flask 1A using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 2.0 ml and discard the solution into a waste bucket. Draw up another 2.0 ml and add to SRP flask 5A. Draw up another 2.0 ml and dispense into flask SRP 5B. Swirl flask to mix.
f. Using a new clean pipette tip repeat the process described above for each of the sediment samples, matrix and standards so that all TP flasks are complete. Between each sample pair use a new clean pipette tip. Swirl flasks to mix well.
g. Run the samples in the flasks according to the TP SOPs listed in section A.3 starting at step 13.

II- **For TN analysis:**

a. Prepare sample and standard tubes as described in section A.5 Total Nitrogen. The same calibration standards are used for sediments as are used with water, however be sure to add 17.0 ml of the digestion blank water to each of the 1Liter volumetric flasks before bringing each working standard up to volume.

b. To minimize the impact of turbidity interference from the digested solution, the sediment digestions are diluted 1:30. For all digested sample culture tubes fill with 29.0 ml of DDI water. Draw up 1.00. ml of the contents in each digestion flask using an an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 1.0 ml and discard. Draw up another 1.0 ml and dispense into the appropriate culture tube. Repeat this procedure with all of the samples digested.

c. Use the vortex mixer to blend the sample. Proceed with analysis as describe in section A.5.

15- Calculations: Calculate TP and TN as described in the appropriate SOP sections but remember to substitute the proper working standard solutions for the respective calculation curves.

a. Sediment Total Phosphorus

$$\text{Sediment TP (mg/Kg dry weight)} = \frac{Ctp \times DF \times Vls}{W}$$

Where: CTP = Concentration of TP in mg/L
DF = the dilution factor (25)
Vtp = volume of extract in liters (0.029L)
W = weight in kg of dried sample

b. Sediment Total Nitrogen

$$\text{Sediment TN (mg/Kg dry weight)} = \frac{Ctn \times DF \times Vtn}{W}$$

Where: Ctn = Concentration of TN in mg/L
DF = the dilution factor (30)
V = volume of extract in liters (0.029L)
W = weight in kg of dried sample

c. Matrix Spikes:

The P matrix spike adds 10 mg/kg{The 10 mg/L Phosphorus Standard Solution / 1000 ml/L = 0.01 mg/ml[(10 ml) (0.01 mg)] /.010 Weight (Kg)= Approximately 10.0 mg/Kg phosphorous}.

The N matrix spike adds 50 mg/kg{The 50 mg/Nitrogen Standard Solution / 1000 ml/L = 0.05 mg/ml[(10 ml) (0.05 mg)] /.010 Weight (Kg)= Approximately 50.0 mg/Kg nitrogen}.

Written by Jeff Schloss and Bob Craycraft
Last Updated on 09/26/05
c:/CFBdocuments/SOPs/LSOP2005SedPN.doc

A.7- Phosphorus Fractionation of Sediments / Loosely-Sorbed and Iron Bound Phosphorus SOP (2005) (Sediment or sludge sample)

Reference: NELAC SOP by Spectrum Analytical Inc. Provided by ENSR; modified and expanded by CFB

Note: use extreme caution when handling the phosphorus analytical glassware. All glassware is to be acid washed (10 minutes in 30% HCL), rinsed with DDI water 6 times and dried with openings facing down on flat drying rack. If there is any doubt of the cleanliness of the glassware, acid wash all applicable materials as described before proceeding any further.

A) **Sample Prepreparation:**

1) Samples to be analyzed should have been collected, iced in cooler, thoroughly mixed and air dried for at least 24 hours. These samples are considered semi-solid and are used in the extraction unless alternative requests have been made (freeze dried, dried at a specific temp and /or milled).
2) If a subsample of the sediment samples have already been dried for 24 hours at 104^{o}C +/- 1^{o}C go to step B)1).
3) The day of the sample analysis (or the day before you want to run this procedure) remove a maximum of 7 sediment samples from the refrigerator. Follow the procedures in SOP A.5.1 for Fixed Solids to determine dry weight basis (percent solids) of each sample.

B) Glassware and Reagents:

1) In the morning of analysis make up the following reagents as described in the following section A.3.1-"Mixing other reagents" of the section A.3.-"Total Phosphorus SOP (2005)":
    a. 5 N H2SO4
    b. 11N H2SO4
    c. 10N NaOH
2) In addition prepare the following solutions:
    d. Ammonium Chloride Solution (1.0 molar): Partially fill a liter volumetric flask with DDI water. Add 53.49 grams ammonium chloride. Adjust the pH to 7.0 with concentrated ammonium hydroxide. Perform this step dropwise. Do not keep the ammonium hydroxide bottle open during this procedure. Dissolve and bring up to volume to 1L with DDI water. Check the pH of the ammonium chloride before each use. (Used for Loosely-sorbed P).
    e. Buffered Dithionite Solution: Add 9.24 grams $NaHCO_3$ and 19.15 grams $Na_2S_2O_4$ to approximately 500 ml DDI water in a 1 liter volumetric flask. Dissolve and dilute to one liter with DDI water. Make fresh daily. (Used for Fe-P)
3) Place the following glassware in the acid bath (30% HCL) for 10 minutes:
    f. 12- 40ml glass centrifuge tubes (Kimble #45200-40) and screw-caps
    g. 24 25mm x 150mm (55ml) test tubes (Corning #9826-25) and screw caps.
    h. 1 Stainless spatula

i. 1 Ceramic spatula with pestle
j. 1 125 ml Erlenmeyer flasks
k. 1ea 50ml, 100 ml and 250 ml graduated cylinder TD
l. 1 1000 ml volumetric flask
m. 1ea 100 ml, 200 ml and 400 ml beakers
n. 1 10 ml graduated glass pipette

C) Loosely-Sorbed P Extraction Procedure

1) Tubes and rack positions are numbered to keep track of samples. Set up the sample batch in the orange centrifuge tube rack in the following manner:

Tube 1-	Blank – leave empty.
Tube 2-	2 mg/L Std. – leave empty.
Tube 3-	Sample 1- Weigh 5.0 +/-0.2 grams of the semisolid Sample 1 into centrifuge tube, write weight of sample onto the datasheet.
Tube 4-	Duplicates of Sample 1, weigh sample one as in Tube 3
Tube 5-	Duplicate of Sample 1 for matrix spike, weigh sample one as in Tubes 3 and 4
Tubes 6 – 11	Samples 2 through 7, weigh 5.0 +/-0.2 grams of each of the semisolid samples into each of these centrifuge tubes.
Tube 12	Blank, leave empty.

2) Make sure you have transferred all pertinent information from the sediment sample label and weight results to the LSP/FEB Phosphorus data sheet at this time (i.e. lake, site, date, depth, etc.).
3) Pour about 20 ml of 30mg/L NIST phosphorus standard solution (Hach #1436716) that has come to room temperature into an acid washed and triple DDI rinsed beaker 50 ml glass beaker. Using a clean 5.0 ml Class A, glass volumetric pipette draw up 5.0 ml of stock standard and dispense the standard into a waste bucket. Subsequently, draw up 5.0 ml of phosphate standard from the beaker and dispense it into Tube 2 (2 mg/L P standard)
4) Using a clean 10.0 ml Class A volumetric pipette draw up 10.0 ml of 30mg/L stock standard from the beaker and dispense the standard into a waste bucket. Subsequently, draw up 10.0 ml of phosphate standard from the beaker and dispense it into Tube 5 that already contains a sediment portion of sample 1 (4 mg/L spike).
5) Pour about 400 ml of buffered Ammonium Chloride solution into a clean 500 ml beaker. Using a clean 50.0 ml graduated pipette draw up about 50 ml of sample and dispense into the waste bucket. Refill the pipette and carefully bring each graduated centrifuge tube up the 25 ml mark. *Note: If you accidentally slightly overfill the tube do not pour any liquid out. Record the actual volume on the data sheet.*
6) Cap all tubes tight and clip the tubes onto the Barnstead Thermolyne Labquake® Rotating Shaker (Model 415110) and set the lab timer for 2 hours.
7) While waiting for shaking to complete during the last half an hour set up the sample flasks and standards for the phosphorus (SRP) analysis:

8) Prepare Sample and Standard Flasks as Follows:
   a. Arrange thirty-two125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 16B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.
   b. Prepare SRP standards and blank as follows (for calibration curve):
      i. Label 7 clean 500 ml, volumetric flasks with the respective standard concentrations:, 500 µg/L, 100 µg/L, 50 µg/L, 10µg/L, 5 µg/L , 2 µg/L and 0 µg/L. Label 1 clean 250 ml volumetric flask as 1000 µg/L.
      ii. Using a 50 ml graduated cylinder add 50 ml of buffered Ammonium Chloride solution into each 500 ml flask and 25 ml of the solution into the 250 ml flask.
      iii. Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/L stock standard, as 30.0 mg/L PO4-3, into the appropriate standard concentrations described in Table 8. *Note: For the last four standards you will utilize the 1000 u/L solution you will have just mixed.*

Table 10. Orthophosphate working standards for LS-P.

Standard Solution (*u* g/L) [1]	HACH Stock Solution (ml) [2]	1000 u/L Solution (ml)[3]	Volumetric Flask Used	Erlenmyer Flask (ID) [4]
1000	25.0	0.0	250 ml	1B
500	25.0	0.0	500 ml	2A
100	5.0	0.0	500 ml	2B
50	0.0	25.0	500 ml	3A
10	0.0	5.0	500 ml	3B
5	0.0	2.5	500 ml	4A
2	0.0	1.0	500 ml	4B
(blank) 0	0.0	0.0	500 ml	1A

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] volume of stock HACH standard (HACH cat # 1436716) added to the designated volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above.
[3] volume of 1000 µ/L calibration working standard added to a 0.5L volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer Flasks into which the respective standards and blanks are placed.

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

   iv. All volumetric flasks are then brought to volume with DDI H2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved.
   c. Set up the yellow tube rack with 12 cleaned culture tubes with caps in row 1.
   d. Turn on the Milton Roy 1001+ Spectrophotometer and let it warm up.

9) After shaking is complete, place tubes into the IEC HN SII (Thermo Electron) centrifuge and spin at 5000 rpm for 15 minutes. Be careful to balance all the samples and start up the

centrifuge bringing the rpms up slowly. Do not use the brake –allow the rotor to spin down on its own.

10) To minimize the impact of turbidity interference the sediment elutants are diluted 1:10. Draw up 5.0 ml of the blank in centrifuge tube 1 using an adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 5.0 ml and discard the solution into a waste bucket. Draw up another 5.0 ml and add to flask 5A. Draw up another 5.0 ml and dispense into flask 5B
11) Using a new clean pipette tip and without disturbing the soil pellet, repeat the process described above for each of the sediment samples, matrix and standards so that all flasks 5A through 16B are complete. Between each sample pair use a new clean pipette tip.
12) Run the samples in the flasks according to the SRP SOPs listed in section A.2 starting at step 7.
13) While waiting for the mixed reagent to react:
    a. Carefully pipette out the remaining sample in each of the centrifuge tubes and transfer the contents of each tube into the respectively marked culture tube in the yellow rack and cap. These samples are held in the refrigerator until you are sure you do not need to rerun this batch on the basis of QC results.
    b. Using the 30% HCL squirt bottle add about 30 ml of acid to the centrifuge tube #1 and fill the cap with acid, let stand for 10 minutes and then rinse 6 times with DDI water and dry inverted.

D) Iron Bound P Extraction Procedure

2) If you have not already done so (step 11 above), without disturbing the soil pellet, carefully discard the supernatant ammonium chloride solution from the previous extraction. Remove as much liquid as possible without discarding any soil sample.
3) Bring up to 25m1 final volume with buffered dithionite solution to each centrifuge tube with sample. This solution must be freshly made. *Note: If you accidentally slightly overfill the tube do not pour any liquid out. Record the actual volume on the data sheet.*
4) Prepare a sample blank by adding the dithionite solution to centrifuge tube #1 bringing it up to 25 ml. Again,
5) Prepare a 10-mgL standard from the 50 ppm phosphate stock standard by adding 5 ml stock standard using a Class A fixed volume 5 ml volumetric pipette that has already been filled once with the 5.0 ml of standard that was discarded into the waste bucket. Bring to 25.0 ml with dithionite solution. See *Note* in step 2) above.
6) Cap all tubes tight and clip the tubes onto the Barnstead Thermolyne Labquake® Rotating Shaker (Model 415110) and set the lab timer for one hour.
7) While waiting for shaking to complete during the last half an hour set up the sample flasks and standards for the phosphorus (SRP) analysis:

   Prepare Sample and Standard Flasks as Follows:

    a. Arrange thirty-two125 ml Erlenmeyer flasks (Corning # 5100-125) alpha-numerically, 1A through 16B, and place the flasks sequentially into the polypropylene sterilizing trays before filling the flasks with samples or standards.
    b. Prepare SRP standards and blank as follows (for calibration curve):

i. Label 6 clean 500 ml, volumetric flasks with the respective standard concentrations: 500 µg/L, 100 µg/L, 50 µg/L, 10 µg/L, 5 µg/L, and 0µg/L. Label 2 clean 250 ml volumetric flasks 2000 µg/L and 1000 µ/L

ii. Using a 25 ml graduated cylinder add 10 ml of buffered dithionite solution into each 500 ml flask and 5 ml into each of the 250 ml flasks.

iii. Using Class A fixed volumetric pipettes dilute pre-purchased HACH (cat #1436716) 30.0 mg/L stock standard, as 30.0 mg/L PO4-3, into the appropriate standard concentrations described in Table 11. For the last three standards you will utilize the 1000 u/L solution you will have just mixed.

Table 11. Orthophosphate working standards for Iron Bound P.

Standard Solution (*u* g/L) [1]	HACH Stock Solution (ml) [2]	1000 u/L Solution (ml)[3]	Volumetric Flask Used	Erlenmyer Flask (ID) [4]
2000	50.0	0.0	250 ml	1B
1000	25.0	0.0	250 ml	2A
500	25.0	0.0	500 ml	2B
100	5.0	0.0	500 ml	3A
50	0.0	25.0	500 ml	3B
10	0.0	5.0	500 ml	4A
5	0.0	2.5	500 ml	4B
(blank) 0	0.0	0.0	500 ml	1A

[1] Standards used to derive calibration curves (prepared within two hours of analysis).
[2] volume of stock HACH standard (HACH cat # 1436716) added to a 500 ml volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above.
[3] volume of 1000 u/L calibration working standard added to a 500ml volumetric flask and brought to volume with DDI water to derive the working standards presented in the leftmost column above. Used for lower standard concentrations.
[4] Erlenmeyer Flasks into which the respective standards and blanks are placed.

Note: orthophosphate standards are not preserved and should be prepared daily no more than two hours prior to the addition of the mixed reagent.

iv. All volumetric flasks are then brought to volume with DDI H2O water, covered with Parafilm and inverted ten times to assure the samples are well mixed and a homogeneous solutions is achieved.

c. Set up the yellow tube rack with 12 cleaned culture tubes with caps in the third row.

d. Turn on the Milton Roy 1001+ Spectrophotometer and let it warm up if it has not been on.

8) After shaking is complete, place tubes into the IEC HN SII (Thermo Electron) centrifuge and spin at 5000 rpm for 15 minutes. Be careful to balance all the samples and start up the centrifuge bringing the rpms up slowly. So as not to disturb the sediment, do not use the brake –allow the rotor to spin down on its own.

9) To minimize the impact of turbidity and matrix interference from the dithionite solution, the sediment elutants are diluted 1:50. Draw up 1.0 ml of the blank in centrifuge tube 1 using an

adjustable Thermo Labsystems Finipipette (cat # 40270-280) set to 1.0 ml and discard the solution into a waste bucket. Draw up another 1.0 ml and add to the culture tube in flask 1A. Draw up another 1.0 ml and dispense into flask 1B.

10) Using a new clean pipette tip and without disturbing the soil pellet, repeat the process described above for each of the sediment samples, matrix and standards so that all flasks 5A through 16B are complete. Between each sample pair use a new clean pipette tip.

11) Run the samples in the flasks according to the SRP SOPs listed in section A.2 starting at step 7.

12) While waiting for the mixed reagent to react:

    a. Carefully pipette out the remaining sample in each of the centrifuge tubes and transfer the contents of each tube into the respectively marked culture tube in the yellow rack and cap. These samples are held in the refrigerator until you are sure you do not need to rerun this batch on the basis of QC results.

13) Glassware cleanup - Immediately rinse out the flasks and other glassware three times with DDI H2O after the run and place the glassware in the drying rack on the Rubbermaid cart. If there is room, place the rinsed glassware into the acid bath and let sit for one hour, otherwise, fill the flasks with DDI H2O and place out of the way until room becomes available in the acid bath. When pulling glassware out of the acid bath rinse three times with DDI H2O and place the glassware upside-down in the drying rack.

E) Calculations:

1) Calculate the measured concentration in mg/L phosphate as described in the calibration curve section of the Soluble Reactive Phosphorus SOP (A.2.13). Be sure to use the specific calibration values appropriate for each SRP analysis from Tables 10 and 11 for Loosely-sorbed and Iron bound P respectively.

2) For Loosely-sorbed P:

$$\text{LS-P (mg/Kg dry weight)} = \frac{Cls \times DF \times Vls \times S}{W}$$

Where:
- Cls = Concentration of Loosely-sorbed P in mg/L
- DF = the dilution factor (5; if steps C9-C10 are followed)
- Vls = volume of extract in liters (0.25L or check datasheet)
- S = percent solids of sample
- W = weight in kg of semisolid sample

3) For Iron Bound P:

$$\text{Fe-Bound-P (mg/Kg dry weight)} = \frac{Cib \times DF \times Vib \times S}{W}$$

Where:
- Cib = Concentration of Fe-bound P in mg/L
- DF = the dilution factor (50; if steps D8-D9 are followed)
- Vib = volume of extract in liters (0.025 L or check datasheet)
- S = percent solids of sample
- W = weight in kg of semisolid sample

4) Matrix Spike Calculation: The spike recovery for the loosely sorbed phosphorus extraction is typically low. During the first extraction procedure the phosphorus is taken up by the iron components found in the sample. The final spike recovery is calculated from the combined recovery of the loosely sorbed and the iron bound constituents. The matrix spike adds 10 mg/kg{The 10 mg/L Phosphorus Standard Solution / 1000 ml/L = 0.01 mg/ml[(10 ml) (0.01 mg)] /.010 Weight (Kg)= Approximately 10.0 mg/Kg phosphorous}.

Written by Jeff Schloss and Bob Craycraft
Last Updated on 09/26/05
c:/CFBdocuments/SOPs/LSOP2005ExP.doc

A.8- Chlorophyll *a* SOP (2004)

Chlorophyll *a* Extraction: goto 2A for periphyton; 2B for filtered lake water

1) Frozen samples will be analyzed within 28 days of collection.

2A) Frozen periphyton filters will be placed in a numerically labeled 15ml glass centrifuge tubes (Kimax model 45166) with approximately 10 mL of cold 90% acetone with $MgCO_3$ buffer.

3A) Periphyton algal cells will be disrupted using an ultrasonic probe (Kimatica Model CH 6010, Kreins, Luzurne Switzerland) set to 30% power level (200 watts) for 15 seconds while held above an ice-bath. Immediately following the cell disruption the tube is placed into the ice bath up to its solution level to insure cooling down of the sample.

NOTE: For the types of algae found on the periphyton plates (as well as samples from sediments) we have found this method to deliver the best yields. A 15 second low power disruption time is sufficient for disruption without causing significant heating of the sample.

2B) Frozen Lake sample filters are placed in a Kimax brand glass mortar with approximately 10 ml of cold 90% acetone with MgCO3 buffer. Carefully use the glass mortar to grind and dissolve the filter do not let high levels of frictional heat to occur.

3B) Pour into a numerically labeled 15ml glass centrifuge tubes (Kimax model 45166). Rinse the mortar with 90% acetone to tranfer all of the residue to the centrifuge tube.

4) The algal samples will be brought to 15 ml volume with 90% acetone with $MgCO_3$, capped and allowed to "steep" refrigerated for 4 hours in the dark.

5) After the 4 hour extraction period the first 8 samples will be placed into the Clay Adams Dynac II centrifuge (model 420103).

6) Turn the centrifuge power button to the "on" position.

7) Make sure the "speed" dial is set to zero before proceeding. The centrifuge speed should be raised slowly, as described below in step 8, to prolong the life of the centrifuge motor.

8) Set the timer to 20 minutes.

9) Slowly raise the centrifuge speed to 1600-rpm (equivalent to 500g).

10) After the 20 minute centrifugation period, place the eight centrifuge tubes in a centrifuge tube rack and immediately place the centrifuge rack in the chlorophyll *a* fume hood under subdued lighting.

11) Turn the centrifuge "speed" dial to zero.

12) Proceed with the chlorophyll *a* analytical procedure as outlined in "Analyzing Samples on the Milton Roy 1001^+ Spectrophotometer (2005)" until you have analyzed the first 8 chlorophyll *a* samples and recorded the data on the chlorophyll *a* "spec notebook" datasheet.

13) Repeat steps 6 through 11 until all samples have been analyzed. Note: when you centrifuge less than eight samples balance the centrifuge with centrifuge tubes filled with "90% acetone without $MgCO_3$" in the remaining slots.

14) The chlorophyll *a* results will be generated using the monochromatic spectrophotometric equation with a pheophytin correction:

$$\text{Chlorophyll } a = \frac{26.7 * (664_b - 665_a) * \text{extract volume (ml)}}{\text{Sample volume (L)} * \text{path length (cm)}}$$

or

$$\frac{26.7 * (664_b - 665_a) * \text{extract volume (ml)}}{\text{Sample Area (m2, periphyton)}}$$

where b = before acidification
a = after acidification

Preparation:

100% Acetone - Purchase Certified ACS Acetone (Fisher Scientific Cat # A18-4) from our scientific supply vendor.

90% Acetone without $MgCO_3$ – Dilute the stock 100% Acetone (above) to 90% Acetone using the following procedure:

a) Wash the 1L volumetric flask and funnel, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.

b) Rinse the 100mL class-A graduated cylinder, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.

c) Fill the class-A graduated cylinder to 100ml with DI H_2O and pour the contents into the 1L volumetric flask.

d) Bring the 1L volumetric flask to volume by slowly adding 100% stock Acetone solution. Note: use the 100% acetone Nalgene squirt bottle to add the final 20-50ml of acetone solution to assure accuracy.

e) Transfer the contents of the 1L flask into the 2.5L amber reagent bottle that is labeled "90% Acetone without $MgCO_3$". Invert the 2.5L reagent bottle 10 times to assure you have achieved a homogeneous mixture.

f) Rinse the 1L volumetric flask, the 100mL graduated cylinder and the funnel with DI H_2O (three times each) before returning the glassware to the chlorophyll *a* fumehood.

90% Acetone with $MgCO_3$ –

g) Wash the 1L volumetric flask and funnel, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.

h) Rinse the 100ml class-A graduated cylinder, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.

i) Fill the class-A graduated cylinder to 99ml with DI H_2O and pour the contents into the 1L volumetric flask.

j) Invert the $MgCO_3$ stock solution 20 times and then add 1ml of $MgCO_3$ stock solution, dispensed via a 1ml volumetric pipette, to the 1L volumetric flask.

k) Bring the 1L volumetric flask to volume by slowly adding 100% stock Acetone solution. Note: use the 100% acetone Nalgene squirt bottle to add the final 20-50ml of acetone solution to assure accuracy.

l) Transfer the contents of the 1L flask into the 2.5L amber reagent bottle that is labeled "90% Acetone with $MgCO_3$". Invert the 2.5L reagent bottle 10 times to assure you have achieved a homogeneous mixture.

m) Rinse the 1L volumetric flask, the 100ml graduated cylinder and the funnel with DI H_2O (three times each) before returning the glassware to the chlorophyll *a* fumehood.

0.1N HCl Solution – *Note: the preparation of the HCL working solution should be undertaken under a fume hood designated for acid preparation.*

a) Fill an acid washed 1000ml volumetric flask with approximately 500ml DI H_2O and place the flask into an ice bath in the fume hood for 15 minutes.

b) Pour approximately 25 ml of 12.1N HCL (FS Cat # A144c-212) into a 50ml acid washed beaker under the fume hood.

c) Draw up 8.3ml of the Stock HCL using a class-A glass pipette and dispense the acid into the 1000ml volumetric flask.

d) Swirl the flask to obtain a homogeneous mixture.

e) Place the 1000ml volumetric flask into a PP tray without ice and let the diluted acid solution come to room temperature.

f) Once the diluted acid solution reaches room temperature bring the volumetric flask to volume by slowly adding DI H_2O from a Nalgene squirt bottle.

g) Parafilm the 1000ml volumetric flask and invert the flask 20 times to assure the 0.1N HCl solution is a homogeneous mixture.

h) Store the 0.1N HCl solution in a PP tray on the lab bench.

$MgCO_3$ Stock Solution – Prepare the $MgCO_3$ stock solution in the designated 250ml amber PP bottle. Discard this solution after 6 months.

n) Rinse the 100ml class-A graduated cylinder, designated for mixing the chlorophyll *a* solvents and stored under the chlorophyll *a* extraction fume hood, three times with DI H_2O.

o) Fill the class-A graduated cylinder to 100ml with DI H_2O and pour the contents into the designated 250ml amber PP bottle.

p) Measure out 1gram finely powdered $MgCO_3$ (FS Cat # M27-500)

q) Add the 1gram MgCO3 to the 250ml amber PP bottle.

r) Invert the 250ml PP bottle 20 times to mix the reagent and subsequently store the reagent under the chlorophyll *a* fume hood until needed.

A.9 Free Carbon Dioxide (2005)

Reference Source: Standard Methods 20th Addition- 4500-CO2 C.

Free Carbon Dioxide Titration:

Free Carbon Dioxide samples should only be taken from the 250ml amber bottles that have been completely filled and sealed or from sealed sample syringes. Use care when pouring the samples since the CO_2 concentration can easily be altered by atmospheric contamination. The procedure for measuring CO_2 is as follows:

1) With a 100 milliliter graduated cylinder, carefully pour out a 100ml sample by letting the water flow down the side of the cylinder without introducing bubbles into the solution.

2) Pour the sample into a beaker and add six drops of phenolphthalein indicator solution.

3) Carefully fill the burette with 0.00227N NaOH titrant from the 250ml amber bottle with the flip top. Flick the burette to dislodge trapped air bubbles. Gently squeeze above the clear glass bead in the tubing to allow the titrant to flow out. Bring the fluid level in the burette to the 0.0 milliliter mark while holding the burette vertically making sure no air bubbles are left in the tip.

4) Titrate until the solution appears a faint pink (pH 8.3) and record the number of milliliters titrant added (1milliliter NaOH titrant = 1 ppm Free CO_2).

Reagent Preparation:

Carbon dioxide-free water: Prepare all stock and standard solutions and dilution water for the standardization procedure with distilled, deionized water that has been freshly boiled for 15 min and cooled to room temperature. The final pH of the water should be ≥ 6.0 and its conductivity should be < 2 μmhos/cm.

0.00227N NaOH: (mix fresh each run) using a volumetric pipette add 22.7 of the 0.01N stock solution of NaOH ml into a 1000ml volumetric flask. Bring volume up to 1000ml.

Phenolphthalein indicator **-** In a 100 ml volumetric flask, dissolve 1 gram phenolphthalein crystals in 100ml 95% ethanol and store the solution in two 60 ml plastic drop dispensing bottles.

A.10 Dissolved Oxygen (Titration; Azide Modification; 2005)

Reference Source: Standard Methods 20th Addition- 4500-O2 B.C.

Winkler Dissolved Oxygen Titration:

Dissolved Oxygen samples should be measured as follows (Note: The first three steps should be undertaken in the field using 300 ml BOD bottles with tapered glass caps):

Fixing the sample:

1) Add 1 milliliter of reagent #1 (MnSO4) by releasing the liquid onto the neck of the bottle (to minimize air bubbles getting into the sample).

2) Add 1 milliliter of reagent #2 (alkali-iodide-azide) by releasing the liquid onto the neck of the bottle (to minimize air bubbles getting into the sample).

3) Stopper the sampling bottle and mix by inverting the bottle ten times. Allow the resulting material to settle until at least one third of the bottle is clear and mix again as described above. Allow the solid material to settle a second time.

4) When 1/3 of the bottle is clear, carefully add 1 milliliter of concentrated sulfuric acid by allowing the stream of drops to flow down the neck of the bottle. Note: Reagent #3 is concentrated sulfuric acid and should be handled with care! Restopper the bottle and invert gently until the solid material has dissolved.

Titration Procedure:

Titrations should always be performed against a white background.

Carefully measure 100 milliliters of your water sample in a graduated cylinder and empty the contents into the beaker. Remember, your water sample contains strong acid and should be handled with care.

5) Carefully fill the burette with Sodium Thiosulfate or PAO from the 250ml amber bottle with the flip top. Flick the burette to dislodge trapped air bubbles. Gently squeeze above the clear glass bead in the tubing to allow the titrant to flow out. Bring the fluid level in the burette to the 0.0 milliliter mark while holding the burette vertically making sure no air bubbles are left in the tip.

6) Slowly add the titrant, drop by drop, to the water sample while stirring with the glass rod. Titrate until the sample reaches a faint yellow color.

7) When the faint yellow color is reached, 6 drops of starch indicator following which the sample will become a shade of blue and you should proceed to Step 8. If the starch indicator is added too soon the sample will turn black and if it is added too late, the sample will remain clear. In either of the latter cases, another water sample should be measured out and step 6 repeated.

8) Continue titrating until solution becomes clear. Record the milliliters of titrant added; calculate your dissolved oxygen concentration in milligrams per liter by multiplying by 2.0.

9) Rinse the beaker, stirring rod and graduated cylinder well between successive samples.

Reagent Preparation:
Oxygen-free water: Prepare all stock and standard solutions and dilution water for the standardization procedure with distilled, deionized water that has been freshly boiled for 15 min and cooled to room temperature. The final pH of the water should be ≥ 6.0 and its conductivity should be < 2 μmhos/cm.

Manganous sulfate solution: Dissolve 480 g MnSO4 4H2O, 400 g MnSO4 2H2O, or 364 g MnSO4 _ H2O in distilled water, filter, and dilute to 1 L. The MnSO4 solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

Alkali-iodide-azide reagent: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

Sulfuric acid, H2SO4, conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

Starch: Use either an aqueous solution or soluble starch powder mixtures.
To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100mL hot distilled water.

Sodium Thiosulfate or PAO solutions (0.025M): are obtained premixed as standard solutions where 1ml = 2 mg/L DO when 100ml is titrated.

Appendix B
Center for Freshwater Biology Standard Operating Procedures (SOPs) for Field Protocols

B1. - Field Measured pH (2005)

Reference: Standard Methods 20th Addition Method 4500H+ and Hanna pH meter manual

Sample requirement: Collected in airtight syringe and sealed or in capped PE bottles, Stored on ice in the dark. Allowed to come to ambient or room temperature before analysis.

The pH meter (Hanna Model HI-9025) is fitted with a Beckman STAR Low Ionic Strength Combination pH probe (Model 39847).

Notes: Calibration procedure should be undertaken before any samples are processed. Always insert the pH and ATC probes 1.5" into the solutions and leave 0.25" clearance from the bottom). The carry case of the meter includes a built in probe holder to facilitate keeping both probes in proper position while in the sample.

A-Calibration:

1) Make sure both the pH probe and the temperature probe are plugged into the pH meter. Remove the cap and the orange side plug from the pH probe before proceeding any further. Add 1N KCl saturated with AgCl electrode filling solution if necessary (the filling solution should be a few mm below the side plug opening). Place the pH electrode and temperature probe in a lakewater sample and let sit for 10 minutes.
2) After the 10 minute "warm-up" period, pour a pH 7.01 buffer solution into a 100 ml plastic beaker. Lift the pH probes out of the water sample, rinse with DDI H2O and place the probes into the pH 7.01 buffer solution.
3) Press the CAL button (the CAL and BUF indicators will be displayed on the LCD screen and the BUF should read 4.01). You want to select a different pH buffer (pH 7.01) which is done by pressing the up arrow or down arrow buttons on the meter until the BUF reads 7.01.
4) Wait 30 seconds or until the LCD display reads READY, and then press the CFM button to confirm the first buffer solution (when the electrode is submerged into the buffer solution, the meter will attempt to stabilize. If the readings fluctuate for more than 10 seconds, the LCD will blink NOT READY. If the reading is stable, READY and CON will blink).
5) Rinse the probes with DDIH2O, pour out a pH buffer 4.01 solution in a clean beaker and submerse the probes into the buffer solution. You should adjust the BUF display to read 4.01 at this point by pressing the down arrow if necessary.
6) Proceed as in step 4. If the calibration symbol (beaker) on the LCD screen blinks then your buffer is contaminated or you used the wrong buffer solution. Repeat step 5 again making sure you are using the correct buffer (4.01).
7) If both the calibration symbol and the probe symbol blink alternately than the slope of the calibration is unacceptable (not within 85%-105%). Try to recalibrate starting from step 2) above. If the slope is still unacceptable you will need to clean or replace the pH probe. See the section below for the probe cleaning instructions (Section D).
8) After successful reading of the second buffer press the CAL buffer to accept the calibration.

9) Once the calibration process is successful, you should rinse the probes with DDI H2O and submerse the probes in a lake-water sample. Let the pH reading equilibrate for approximately 10 minutes and than begin analyzing your samples.

B-Running Samples:

1) Dispense approximately 50 ml of sample into a clean beaker being careful to fill the beaker slowly allowing the sample to run down the side of the beaker with little agitation. Insert the probes and let the pH reading stabilize for two minutes (or until the readings are stable to the nearest 0.01 unit for 10 seconds). Record your reading on the field data sheet and dispense the remaining sample for the replicate reading. Proceed to the next sample, rinsing with DDI H2O between samples. And repeating the process until all samples are read.

C-Calibration Check:

1) After running all samples fill a cleaned beaker with fresh pH 7.01 buffer and record the reading on the field datasheet.

D-Probe (Bulb) Rejuvenation:

NOTE: If step one rejuvenates probe, do not proceed to step two. Likewise, if step 2 works, don't proceed to step 3

1) Soak bulb in 1M HCl for one hour. Rinse thoroughly with deionized water.
2) Soak alternatively in 1M HCL and 1M NaOH for one minute in each solution, cycling three times. Then soak in pH 4 buffer for 1 hour
3) Clean bulb with 50/50 mixture of acetone and isopropyl alcohol

Note any instrument or probe discrepancies in the field log book and transfer the information logged into the field equipment log book upon return to the lab

Written by Bob Craycraf t and Jeff Schloss
Last Updated on 07/07/05
c:/CFBdocuments/SOPs/FSOP2005pH.doc

B2.- Field Measured Total Alkalinity (2005)

Reference: Standard Methods 20th edition: 2320 B. (low alkalinity) -modified

This method has been modified in two respects:

1- The ecoregional character of NH lakes makes using a more dilute acid result in the higher sensitivity required to obtain adequate results. Thus, a titrant of .002N H2SO4 is used instead of the .02N acid of standard method.
2- While lab analysis typically uses a pH meter, for the field we use a pH indicator solution for efficiency unless the water has high organic color. A mixed bromocresol green-methyl red indicator allows for a sharper equivalence point at the lower pH that the test requires. It is greenish-blue at pH 5.2, light blue at pH 5.0, light gray at pH 4.8, and light pink at pH 4.5.

Upon special request the protocol can be followed using the Hanna Model HI-9025 pH meter instead of indicator solution. Follow the protocols outlined below but skip step C.2. and substitute pH 4.8 for gray endpoint and pH 4.5 for pink endpoint.

NOTE: This method assumes the water pH is below 8.3 units as occurs in most NH waters. Phenolphthalein indicator can be used to determine this (no pink when added) or a pH reading by meter can be done.

A- In-lab Stock and Working Solution Preparation:

1) Stock Titrant (0.1N) – Pipette 2.8 ml concentrated H2SO4 (app. 36N) into a 1 Liter flask and bring to volume with DDI H_2O
2) Dilute Titrant (.002N) – Pipette 20 ml (+or- depending on the Stock titrant normality as determined below) into a 1 Liter flask and bring to volume with DDI H_2O
3) Calibration Solution: Dry 3 to 5 grams Na2CO3 at 250oC for 4 hours and cool in a dessicator.
    a. Weigh 2.5 +or- .2 grams and transfer into a 1 liter volumetric flask. Record the weight.
    b. Fill the flask with CO2 free (boiled for double distilled water and mix (do not keep this solution longer than one week). This solution is approximately 0.05N.
    c. From the 0.05N Stock solution take 40.00 ml and place in a beaker
    d. Add 60ml CO2 free DDI H_2O and place on a magnetic stirrer (make sure the stirring bar has been washed thoroughly prior to placing it into the beaker to avoid contamination)
    e. Fill a 25.0 ml burette with stock alkalinity titrant (0.1N) making sure no air pockets or bubbles remain in the burette.
    f. Slowly titrate to a pH of about 5. Lift out the pH and temperature electrodes, rinse the electrodes into the beaker and boil gently for 3-5 minutes under a watch glass. Cool to room temperature, rinse the cove glass into the beaker and finish titrating to the pH inflection point. Calculate Normality (all data should be recorded on the corresponding data sheet):

$$\text{Normality} = \frac{\text{grams Na2CO3 in 1L flask x ml Na2CO3 solution taken for titration}}{53 \times \text{ml titrant(0.1N H2SO4) used}}$$

4) Working Solution: Once the Normality of the stock H2SO4 solution is determined 10 Liters of .002N alkalinity titrant should be produced by pipetting 20 ml (+or-) into a 1 Liter flask and bringing to volume with DDI H_2O. Repeat this step until 10 Liters of alkalinity titrant are produced and stored in a 10 Liter Carboy. Make sure the date and lab technician name are included on the carboy label.

B- Alkalinity Field Kit

1) Check Contents of Field Kit for all items included and their condition before departing:
    a. 100 ml plastic graduated cylinder
    b. 150 ml plastic beaker or cup
    c. field burettes (plastic volumetric pipette fitted with ball valve and tip)
    d. glass stirrers
    e. Indicator solution drop bottle- (Brom Cresol Green Methyl Red)
    f. Titrant Solution dispensing bottle (.002N H_2SO_4)
2) Check expiration dates of all chemistry and refresh with new solutions if required.

C-Total Alkalinity Field Measurements:

NOTE: Titrations should always be done against a white background and away from any colored reflections
The graduated cylinder, beaker and stirring rods should be rinsed well between samples.
Allow samples to come to room temperature or ambient temperature within their sampling container before processing.

1) Carefully transfer (without agitation) 50 ml or 100 ml of the water sample (from sample bottles or syringes) into the graduated cylinder. Use 50 ml when you expect Alklainity to be higher than 15 mg/L or when you have a limited amount of sample. Use 100 ml when low alkalinity is expected, Then transfer sample carefully into plastic titrating cup/beaker.

2) Add 8 (for 50 ml sample) or 12 (100 ml) drops of indicator (bromocresol green/methyl red) from the drop dispensing bottle. Stir gently with glass rod.

3) Insert tip of plastic titrant bottle into top of plastic field burette. Squeeze bottle carefully and fill burette with 10-12 ml of titrant (0.002 N H 2 SO 4).

4) Hold burette with one hand so that thumb and forefinger can squeeze above the glass bead in the rubber tubing. Be sure to squeeze gently just behind the bead so you release titrant but do not move the bead down the tube.

5) Clear all air bubbles from the burette by gently tapping the pipette while you hold the burette up and down. Clear any trapped air in the tip area by bending the tube so the tip is pointing up and allowing some titrant to flow out of the tip to release the air. Refill the burette if necessary to bring the titrant level to the zero mark and allowing the released titrant to collect into your waste field container. **DO NOT PUT ANY TITRANT BACK INTO THE TITANT BOTTLE AT ANY TIME.**

6) Add titrant slowly, drop by drop, while stirring with other hand. Titrate until water loses blue coloring and becomes a dull gray color . Record number of milliliters used to reach this first endpoint. Record to the nearest tenth (eg. 5.4 ml); each small line on the burette is 0.1 ml, each half line is 0.5 ml, and each full line is marked with the whole ml number of solution dispensed.

7) -Continue titrating until solution becomes a very faint pink. At this point in the titration, the water will become "pinker" as more titrant is added, so it is very important to titrate only until the first signs of loss of grey and appearance of light pink! Again, record the total number of mls used to reach this second endpoint (in other words, since you began titrating, not since the gray endpoint). For example, if it took 5.4 ml to reach the gray endpoint, plus, 0.4 ml to reach the pink endpoint, the total ml used for the second endpoint would be 5.8 ml. Make sure both endpoints are included on the data sheet.

8) Calculations: We have found that Total Alkalinity results slightly vary due to method used. For comparison to alkalinities derived from Acid Neutralizing Capacity Gran Titrations (pH inflection point determinations) the grey endpoint (pH 4.8) compares the best. For comparisons to historic measurements made with Methyl-Orange indicator (since shown to influence sample pH and no longer used) the pink endpoint (pH 4.5) is best. To compare to the Standard Method low alkalinity method or

the EPA dual endpoint method calculate each (grey and pink) of the alkalinities using equation A and use the second formula (Equation B) below.

$$\textbf{A- Total Alkalinity (mg } CaCO_3\textbf{/L)} = \frac{\textbf{T x N x 50,000}}{\textbf{ml sample titrated}}$$

where: T is the ml of titrant for the endpoint used
N is the normality of the titrant

Calculate both the grey endpoint and pink endpoint alkalinity and add to the field datasheet. If .002N acid is titrated into 100 ml of sample then each 1.0 ml of titrant used would be equivalent to 1.0 mg $CaCO_3$/L. Thus, the burette reading of titrant used is the Total Alkalinity. If 50 ml of sample is titrated with the same titrant used multiply the burette of titrant used by 2.0.

B Total Alkalinity (mg $CaCO_3$/L) = (2 x G) – P

where: G is the grey endpoint alkalinity
P is the pink endpoint alkalinity

Written by Bob Craycraf t and Jeff Schloss
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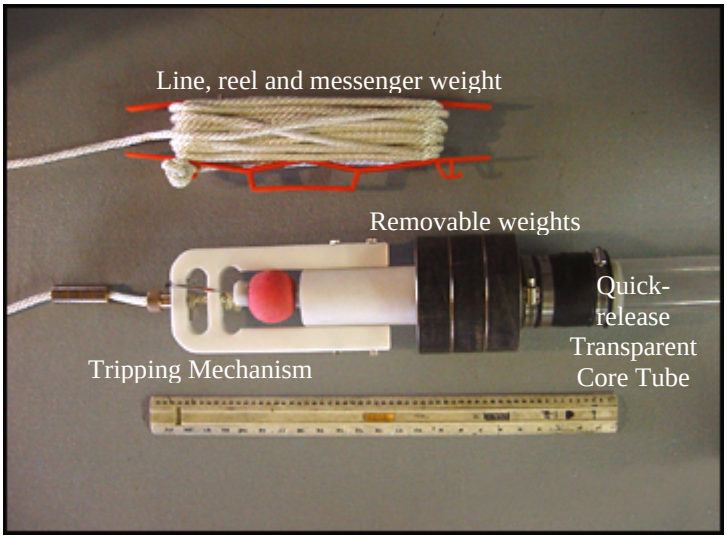
c:/CFBdocuments/SOPs/FSOP2005Alk.doc

B3.0- Use of KB style Core Sampler (2005)



1) Insure that core tube is attached securely to the core head by pushing it fully into the flange.

2) With the unit upright push down on the brass fitting so that the tripping pins open.

3) While still applying pressure to the fitting pull up on the nylon filaments that are attached to the ball seal and place the loops so that they are secured around the tripping pins and release the brass fitting so that the loops are held.

4) Carefully place the unit into the water holding the line reel in one hand and the just above the corer and messenger in the other. Unreel enough line for the depth you are at careful that it will be able to run out freely.

5) If over deep water allow the unit to free fall and penetrate the sediments being careful control the line looslely so that apfter penetration of the sediments the corer will remain upright.

6) Send the messenger down the taunt line and wait for feeling the vibration of the tripping mechanism to occur.

7) Carefully pull the sampler out of the sediments, bring the sampler up to the surface in a smooth manner and just before surfacing the instruments cap the bottom end of the sampler while still under water using the # 11 rubber stopper.

8) Bring the sampler into the boat maintaining it upright, position it over the collection tray and carefully remove the core tube from the core head by smoothly turning the core tube back and forth while maintaining a study down pressure and while someone else is holding the core head stationary. Make measurements of the core depth, notate any observations in the field log books and on the field data sheets and take any photographs of the sediment layering before proceeding.

9) Carefully siphon out the water above the sediment water interface using a siphon tube consisting of a plastic 10 ml volumetric pipette attached to a length of tubing. Keep the pipette tip below the water level but away from the sediment. Depending on the project discard or save the siphoned water sample as directed

10) .If the core is not to be transported intact, carefully remove the bottom stopper and collect the full core or a series of sediment layers as directed by the project specifications. Continue to collect the remaining sample as directed by the project specifications.

11) Before any additional sampling is to continue wash out the core head and core tube with lake water and then rinse with distilled water.

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c:/CFBdocuments/SOPs/FSOP2005KBC.doc

B.4.0- Use of the Interstitial Pore Water Sampler (2005)

1) Insure that the delrin (white) probe point moves freely up and down to cover and uncover the small sampling holes. If not remove the two side screws, clean the contact areas and reassemble.
2) Assemble the probe with enough sections to accommodate the depth of the water and the freeboard of the boat you are sampling from. You should allow for the sample penetration depth and have the upper end of the sampler a minimum of 0.5 meters above the water's surface. During assembly make sure you also inset a clean sampling tubing into the sampler so it is even with the sampler bottom and passes through the center opening of each section without twists or pinching..
3) While over the sampling site carefully insert the probe into the water and hold when you reach the bottom sediment.
4) Taking note of the water level against the sampling tube markings insert the sample into the sediments to the target penetration depth and an additional 5cms.
5) To facilitate penetration in harder sediments you can twist the sample clockwise as you apply penetration pressure. If necessary you can attach the sliding hammer assembly to the top of the tube and hammer in the probe
6) Once the penetration is complete carefully raise the outer tube of the sampler about 5 cm (or until you feel the first sign of resistance. A slight counterclockwise twist will lock the sampler open.
7) Press down on the sampling tubing to insure it is located at the bottom of the sampler. Secure the tubing to the top of the sampler with vinyl tape so it will remain in place as you sample.
8) Wait about 5-10 minutes for the water to equilibrate in the sampler.
9) Using the portable peristaltic pump attach the sampling tubing to the connector marked "in" and begin pumping out the water in the sampler at the lowest speed noting the start time of the pumping. Collect the sample in the graduated collection bottle.
10) If the sampler is purged of water note the volume and time of collection. If the sampling flow remains consistent then allow for two complete exchanges of the sampler.
11) Allow the required time for the sample to come to equilibrium again using the volume and time measurements made previously. Empty the sample container, turn on the pump and then collect sample into the sample container.
12) Continue these steps until you have collected the required amount of sample.
13) If you are using a hand vacuum pump or syringe in lieu of the peristaltic pump be sure to break the vacuum between sampler purging and collection.
14) After sampling is complete be sure to clean the sampler, check the probe point interface and replace the sampling tubing.

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c:/CFBdocuments/SOPs/FSOP2005IPWS.doc

B.6 Specific Conductivity (2005)

Reference Source: Standard Methods 20th Addition- 2510.

Instructions for YSI Model 30 Specific Conductance Meter:
Measurements may be made by submerging probe into the lake or stream or by placing probe into a sample previously collected.

Initial Meter Check and Calibration (before leaving for field sampling):

1. Turn the meter on by pressing the ON/OFF key. The meter will display all segments of the display screen for a few seconds, followed by a self-test. If the meter is not functioning properly, an error message (LoBat, #Err, Ovr or Undr) will be displayed.
2. If the "°C" is not flashing on and off, press the MODE key until it does. This puts the meter into the temperature compensated mode.
3. Rinse the probe with DI water and blot dry with a Kimwipe.
4. Submerge the probe in the 100 (for lake sampling) or 200 (for stream sampling) µS conductivity standard solution and allow to stabilize for two minutes. Ensure there is enough solution to cover the top opening of the probe.
5. Record the Initial Specific Conductance Calibration Value on the Field Data Sheet. Ensure there are no air bubbles on or inside the probe. A 10% error (a reading of 90-100 µS for the 100 µS standard or a reading of 180-220 µS) is acceptable. If the readings are outside of this range then have the laboratory manager recalibrate the unit.
6. Rinse the probe with DI water and return it to the storage chamber.

Field Sampling Procedure:

1. Press the ON/OFF key to turn the meter on. If the "°C" is not flashing on and off, press the MODE key until it does (this puts the meter into the temperature compensated mode). If using a different meter, ensure that it is in specific conductivity mode.
2. Rinse the probe with DI water and blot dry with a Kimwipe.
3. Immerse the probe in the lake, stream or sample container and make sure it is deep enough to cover the entire probe. Do not allow the probe to touch any solid object or the bottom of the container while you are taking readings. It is also important that there are no air bubbles on/in the electrode. To dislodge any bubbles, gently move the electrode through the water before recording the measurement.
4. Agitate by slowly moving the probe back and forth in the sample for a minimum of two minutes for the temperature and specific conductance readings to stabilize. Record the conductivity reading on the Field Data Sheet.
5. Rinse the probe and return it to the storage chamber between measurements. Please turn the meter off when not in use to conserve battery power.
6. At the end of the day, recheck the meter with the standard and record the value in the "Final Calibration Check" section on the Field Data Sheet

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Last Updated on 05/21/06
c:/CFBdocuments/SOPs/FSOP2006/SPCD.doc

B.7 Stream Flow Measurements

Reference: YSI Son-Tek Users Manual.

As the use of this instrument is technical in nature all field crew will need to be trained and certified to use this instrument for any project. An abridged copy of the technical manual will be use for training purposes. The information below summarizes the major steps and references the manual sections used.

The basic operation of the "Flow Tracker ADV" system includes (and manual section reference):

Preparing the system for data collection in the field:

1. Run pre-deployment diagnostic before leaving for the field (sections 3.3 and 6.5.4)
2. Vent the display controller (3.1)
3. Mount the probe on the self setting wading rod (8.1)
4. Perform Filed diagnostics (3.3.)2
5. Set system parameters (2.4)
6. Start data collection in "Discharge" mode (5.)
7. Set your station areas (stream transect points) along a tag line consisting of a fiberglass measuring tape marked in fractional meters that has been anchored to run perpendicular to the water flow (5.2.1).
8. For shallow station readings use the one point method (0.6 * depth, velocity reading) for deeper stations use the two point methods (0.2 and 0.8* depth, velocity readings).
9. Set your system parameters for sampling along the transect.
10. Start your data collection by pressing 3 from the Main Menu START DATA RUN (5.3.2)
11. Place the probe at the required depth using the wading rod vernier. Keep the probe X direction perpendicular to water flow. Press MEASURE to start. Enter station depth and distance along the tag line and watch for velocity reading. Press 1 to accept that measurement or 2 to repeat the measurement.
12. If there is more than one measurement (two-point method) adjust the probe to the second required depth as indicated on the readout and press MEASURE.

13. When the station is complete the unit will display the next station number and predict the location, depth and method if a multiple measurement was use it will start at the last reading fractional depth. You can use the SET LOCATION or SET DEPTH buttons to edit or correct this data.
14. Continue pressing the MEASURE key until all readings are taken.
15. At the final reading (stream edge) press the END SECTION key
16. You can review and edit the collected data by pressing the NEXT STATION and PREVIOUS STATION keys.
17. When all values are checked and edited press the CALC. DISCHARGE key to complete the calculations and close the data file.
18. Press 9 to return to the MAIN MENU. **Always return to the main menu before turning the system off to ensure all data collected has been saved**
19. The Flowtracker (section 6.0) software is used to download the collected data to our lab computers. Be sure to download all data from the unit before returning to the field.

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c:/CFBdocuments/SOPs/FSOP2006/YSIFlow.doc

B.7 YSI 6600 Multiparameter Profiling

Reference YSI 6600 Users Manual and YSI 650 MDS Users Manual.

As the use of this instrument is technical in nature all field crew will need to be trained and certified to use this instrument for any project. An abridged copy of the technical manual will be use for training purposes. The information below summarizes the major steps for measurements.

1. Before leaving for the field the instrument and the probes are fully inspected for fouling and damage. All probes are calibrated in the laboratory before field deployment. Battery levels for both the model 6600 sonde unit and the YSI 650 MDS data display/logger are checked to insure full charges.
2. Before deployment the units are kept out of direct sunlight and the power is turned on for at least 15 minutes. At each site the unit is calibrated for dissolved oxygen, depth and zero fluorescence as directed in the manual.
3. The calibration cup is replaced with the sensor guard cover before deployment and all connections are checked.
4. The datalogger is set to record at measurement intervals between 10 and 15 seconds. After allowing the unit to equilibrate at the surface for 2-5 minutes the 650 MDS is set to start logging and the unit is slowly lowered into the water at a slow rate so readings are logged at approximately every 0.2 meters or so. Lowering can be slowed when the display unit indicates the sonde is traveling through the thermocline to allow for a slower response by the DO and pH sensors.
5. As the bottom is approaching care should be taken to limit movement on the boat so as not to allow the unit to disturb the bottom until all readings are taken.
6. If the bottom is disturbed raise the sonde slightly and allow for the water to settle, watch the display for indication that this has happened.
7. Raise the sonde at the same rate as it was lowered to collect a second set of profile readings from the sonde.
8. When the sonde is raised to the surface replace the sensor guard cover with the calibration cup and store the unit and datalogger out of direct sunlight.

9. Upon returning to the lab take measurements of pH, redox and conductivity standards and note any discrepancies in the equipment logbooks. Wash the units and cables thoroughly before storage.
10. Data can be downloaded from the data logger using the Ecowatch Software.

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c:/CFBdocuments/SOPs/FSOP2006/YSI6600.doc

Appendix C

Standard Operating Procedures for PAR Field Measurements
UNH Center for Freshwater Biology

Updated 5/21/06 by Jeff Schloss and Bob Craycraft

LI-1400 DataLogger PAR Measurements

Standard Operating Procedures

Overview

The LI-1400 DataLogger and calibrated quantum sensors provide the capability to quantify Photosynthetically Active Radiation (PAR) both above and below the water surface. The 'air' sensor remains above the water and quantifies downwelling radiance from the sun each time a discrete measurement is taken; this is most often used to normalize readings taken over several minutes to a constant downwelling value. The 'underwater' sensor is deployed on a frame that is lowered into the water. This sensor is generally used to measure a profile of in-water irradiance versus depth so as to estimate the diffuse attenuation coefficient (K_d), a measure of the rate at which photosynthetically active radiation is attenuated as it passes down through the water-column.

In general, the attenuation of light is exponential versus depth. To obtain K_d from a series of light readings with depth, a series of measurements at at least 8 depths is desired (in shallow waters this may not be possible). One obtains K_d by making a linear regression of sample depth versus ln (PAR) and calculating 1/(slope of the regression). Additional information of interest includes the percent of surface radiation reaching the bottom.

Estimates of K_d are considered robust if the r^2 of the regression is >0.95 (generally >0.98). The precision of the method is estimated by taking 3 complete profiles sequentially and calculating the standard error (SE) of the measurement. The SE should be less than 10%.

Before First Sampling of the Day

1. Insure that the sensors are securely attached to their frames and confirm that the calibrations factors stored in the DataLogger are correct for the sensors in use.
2. Hook up the Underwater BNC connector to Channel I1 labeled "underwater".
3. Hook up the Air BNC connector to Channel I2 labeled "air".
4. Turn the DataLogger 'ON'.
5. Under View, press 'ENTER' to view new data.
6. The first view should say "I1I" which corresponds to the underwater connector. "I2I" corresponds to the air connector.
7. Switch to view I2I, take the cap off of the air sensor, check the reading then cover the sensor with your hand to confirm the reading changes (the reading should decrease with a decrease in light).
8. Switch to view I1I, take the protective covering off of the underwater sensor, check the reading and then cover the sensor with your hand to confirm the reading changes (again the reading should decrease).

At Each Station

1. Turn on the DataLogger.
2. Take out the respective data sheet for the site. Record the time when the underwater sensor is put in the water.
3. Lower the sensor to 10cm. Allow the reading to stabilize (1-2 seconds) and then press 'ENTER'. This logs the data into the DataLogger. Cross off 10cm (and each subsequent depth for which you log data into the DataLogger) on the data sheet.
4. Lower the sensor to the next depth. In shallow areas, record measurements every 25cm as marked on the cable. In deep and/or clearer water areas the sensor can be lowered every 50cm. At least 6-8 depths should be recorded in the DataLogger for each station.
5. When (If) the sensor reaches bottom, write the bottom depth (approximate using the depth markers) on the datasheet and press 'ENTER' to log data into the DataLogger.
6. Raise the underwater sensor out of the water and put the protective cover on. Put the cap on the air sensor also. Turn the DataLogger 'OFF' until reaching the next station.

At End of Sampling

1. Unplug the BNC connectors from the LI-1400.
2. Rinse underwater sensor, frame and cable with freshwater and let dry before storage.

Download Data to Excel in the Laboratory

1. After returning to the lab the data should be retrieved from the DataLogger.
2. Attach the DataLogger to the computer using the serial cable.
3. Open the LI-1400 program and then turn the DataLogger on.
4. Under the remote menu click on 'CONNECT'. Under the connect window, type '2' next to com port number and click 'CONNECT'.
5. Under the remote menu click 'RECEIVE DATA'. Save the data on the computer.
6. Open Microsoft Excel and then open the file you just saved. The file is a delimited file and click 'FINISH'.
7. Download LiCor Data into a new Excel file and **Save As** MPWAP Raw Light Profile (MMDDYY) where the MMDDYY represents the sampling date.
8. Once you are certain that you have successfully downloaded and saved the data the data in the DataLogger should be cleared from memory. This can be done 2 ways:
    a. On the DataLogger, press the 'FCT' key. Arrow to the right twice till clear memory is in the window. Arrow down to clear all, down to date, down to time, and down to clear memory yes/no. Confirm that "clear memory yes" is in the window and then press 'ENTER'. (This may not clear the memory).
    b. In the LI-1400 program, under the remote menu click 'CLEAR DATABASE'. In the clear database window confirm that all is chosen then click 'OK'.

9. Under the remote menu click 'DISCONNECT'. Unplug the DataLogger from the computer, turn it off, make sure that there is no dirt or salt on it and put it away.

Data Processing

1. **Open** *Light Profile Master* Excel File and save the file as MPWAPAttenuation (MMDDYY)

2. Cut and paste the raw light data into the appropriate rows/columns in the MPWAP *Light Attenuation (MMDDYY)* file making sure to separate each station as indicated by the station labels in column A.

3. Edit the depths Column E so that they reflect the correct depths at which each of the readings at a particular station was taken.

4. Run individual regression analyses for each of the light profiles as follows (using APL as an example):

    a. Click on **Tools**, **Data Analysis**, **Regression**
    b. Select 'Input Y Range' as the range of measured Depth [m] in Column E
    c. Select 'Input X Range' as the range of calculated Quantum [LN] in Column H
    d. Select 'Output Range' as the Yellow Shaded Block in Column J
    e. Select 'OK'; this should insert the regression statistics to the right of the data [Note: Do not include data for which the Quantum [Raw Water] data is < 0.1]
    f. Save File (intermediate save so as to not lose data)

5. At this point, the Diffuse Attenuation Coefficients ($\mathbf{K_d}$) should have been calculated for each station at which you did the regression. $\mathbf{K_d}$ = 1/x-coefficient from the regression.

6. QA/QC: Examine the regression output data:

    a. Acceptable regressions must have an $R^2 > 0.95$ and, for stations with an optimal number of sample depths (>8) should have an $R^2 > 0.98$.
    b. Examine the Quantum [Raw Water] data. These data should show a continuous decrease with depth except for the odd cases where the Quantum [Air] data increased significantly from the preceding reading (e.g. the passing of a cloud). Highlight any questionable reading by applying an 'Orange' fill to the cells in question.

7. In the event that the $R^2 < 0.95$ and there is a data point at the top or bottom of the profile that is clearly bad (these are the most likely places for this to occur because of surface reflection or sediment resuspension) you may choose to run the regression again omitting the suspect data. In such cases it is imperative that you make a notation in Column I, just below the $\mathbf{K_d}$ calculation block.

Appendix D-1

Quality Assurance Plan
UNH Water Quality Analysis Laboratory
(Natural Resources)

QAPP for the Water Quality Analysis Lab at the University of New Hampshire, Department of Natural Resources, Durham, NH.

I. Laboratory Organization and Responsibility

Dr. William H. McDowell - Director

Jeffrey Merriam – Lab Manager/QA manager. Mr. Merriam supervises all activities in the lab. His responsibilities include data processing and review (QA review), database management, protocol development and upkeep, training of new users, instrument maintenance and repair, and sample analysis.

Jody Potter – Lab Technician. Mr. Potter's responsibilities include sample analysis, logging of incoming samples, sample preparation (filtering when appropriate), daily instrument inspection and minor maintenance.

All analyses are completed by Jody Potter or Jeffrey Merriam, and all data from each sample analysis batch (generally 40-55 samples) is reviewed by Jeffrey Merriam for QC compliance. All users are trained by the lab manager and must demonstrate (through close supervision and inspection) proficiency with the analytical instrumentation used and required laboratory procedures.

II. Standard Operating Procedures

Standard Operating Procedures for all instruments and methods are kept in a 3-ring binder in the laboratory, and are stored electronically on the Lab manager's computer. The electronic versions are password protected. SOPs are reviewed annually, or as changes are required due to new instrumentation or method development.

III. Field Sampling Protocols

Sample collection procedures are generally left up to the sample originators, however we recommend the guidelines described below, and provide our field filtering protocol on request.

All samples are filtered in the field through 0.7 um precombusted (5+ hours at 450 C) glass fiber filters (e.g. Whatman GF/F). Samples are collected in acid-washed 60-mL HDPE bottles. We prefer plastic to glass as our preservative technique is to freeze. Sample containers are rinsed 3 times with filtered sample, and the bottle is filled with filtered sample. Samples are stored in the dark and as cool as possible until they can be frozen. Samples must be frozen within 8 hours of sample collection. Once frozen, samples can be stored indefinitely (Avanzino and Kennedy, 1993), although they are typically analyzed within a few months.

After collection and freezing, samples are either hand delivered to the lab, or are shipped via an over-night carrier. Samples arriving in the lab are inspected for frozen contents, broken caps, cracked bottles, illegible labels, etc. Any pertinent information is entered into a password protected database (MS Access).

We do not require chain of custody paperwork unless a specific project requires it. If a project requires chain of custody, forms are provided by the specific project's manager.

IV. Laboratory Sample Handling Procedures

Samples are given a unique 5-digit code. This code and sample information including name, collection date, time (if applicable), project name, collector, logger, the

date received at the WQAL, sample type (e.g. groundwater, surface water, soil solution) and any other miscellaneous information, are entered into a password protected database. From this point through the completion of all analyses, we use the log number to track samples. Log numbers are used on sample run queues, spreadsheets, and when importing concentrations and run information into the database

After samples are logged into the WQAL, they are stored frozen in dedicated sample freezers located in the laboratory. Samples from different projects are kept separated in cardboard box-tops, or in plastic bags. Samples that may pose a contamination threat (based on the source or presumed concentration range) are further isolated by multiple plastic bags, or isolation in separate freezer space. This is typically not an issue as we primarily deal with uncontaminated samples.

We do not pay special attention to holding time of samples, as frozen samples are stable indefinitely (Avanzino and Kennedy, 1993). However, we do keep track of the date samples arrive at the WQAL, and can report holding times if necessary. After samples are analyzed they are returned to the project's manager for safe keeping or they are held for a period of time at the WQAL to allow necessary review and analysis of the data by the interested parties (not from a laboratory QC sense, but from a project specific viewpoint). Once the data is analyzed by the project's manager(s), the samples are returned or disposed of, based on the preference of the project's manager.

Chain of custody is only implemented when required by a specific project. This is usually only when it's required by the funding agency, or if the samples could be the basis for an enforcement action.

Samples that arrive unfrozen, with cracked bottles/caps, or with loose caps, are noted in the database and are not analyzed. These samples are disposed of to prevent accidental analysis. The sample originator is notified (generally via e-mail) of which samples were removed from the sample analysis stream. Similarly, if while in the possession of the WQAL, a sample bottle is broken or improperly stored (e.g. not frozen), the sample is removed and the sample originator is notified.

V. Calibration procedures for chemistry

Calibration curves are generally linear, and are made up of 4-7 points. A full calibration is performed at the beginning of each run (a run is generally 40-60 samples) with a reduced calibration (3-5 points) performed at the end of the run. Occasionally calibration data is best fit with a quadratic equation, and this is used if it best describes the data within a specific run.

Standards are made from reagent grade chemicals (typically JT Baker) that have been dried and are stored in a dessicator. Working stock solutions are labeled with the content description, concentration, initials of the maker, and the date the stock solution was made. Generally stock solutions are kept less than one week; however some stocks (Br, Na, Cl, C for DOC) can be stored for several months. Standard solutions are kept for less than one week from the date they were made. Stocks and standards are stored tightly covered, in a dark refrigerator.

Control charts are prepared and printed every few months. However data from each run are looked at within days of analyses. Calibration curves, Laboratory Duplicates, Lab Fortified Blanks (LFB), Lab Fortified Sample Matrices (LFM) and Lab

Reagent Blanks (LRB) are reviewed and are checked against known concentrations (where applicable) to ensure QC criteria are met for each run of samples.

VI. Data Reduction, validation, reporting and verification

Data reduction and validation are performed in a spreadsheet (MS Excel). The Raw data page of the spreadsheet lists the date of analysis, user, analysis performed, project, any issues or problems noted with the instrument on that date, and the sample queue and the raw data exported from the instruments. Most raw data is exported as an area or an absorbance value. A second page (typically named "Calculations") is added to the spreadsheet where known concentrations of standards, check standards and reference solutions are added. The calibration curve(s) is calculated and the concentrations are calculated on this page. Calculated concentrations for all standards, LFB, LFM and IPC are compared to the "known" or prepared values. If these are acceptably close (+/- 10% of the "known") no further changes to the calculated concentrations are made. If there is evidence of drift in the response of the instrument during a run, we try to correct for the drift using the responses from the front end calibration curve and the set of standards analyzed at the end of the run. All reference solutions and replicates must meet certain QC criteria (described below) for a run to be accepted.

Data are then exported to the WQAL database. Exported information includes the unique 5-digit code, calculated concentration, the analysis date, the user, the filename the raw data and calculations are saved in, and any notes from the run regarding the specific sample. Data are sent to sample originators upon completion of all requested sample analyses and following review by the WQAL lab manager. Generally the data include

the 5-digit code, the sample name, collection date, and concentrations, in row-column format. Any information entered into the database can be included upon request. Data transfer is typically via e-mail or electronic medium (CD or floppy disk).

All data corrections are handled by the lab manager. Corrections to data already entered into the database are very infrequent. Typically they involve reanalysis of a sample. In this case, the old data is deleted from the database, and the new value is imported, along with a note indicating that it was re-analyzed, the dates of initial and secondary analysis and the reason for the correction.

Hand written or computer printed run sheets are saved for each run and filed, based on the project and the analysis. Spreadsheet files with raw data and calculations are stored electronically by analysis and date. Information in the database allows easy cross-reference and access from individual samples to the raw data and the runsheets. This provides a complete data trail from sample log-in to completion of analysis.

VII. Quality Control

All analyses conducted at the WQAL follow approved or widely accepted methods (Table 1).

Quality Control Samples (QCS) (from Ultra Scientific) are analyzed periodically (approximately every 20 samples) in each sample analysis batch to assure accuracy. The response/unit concentration is also used to monitor day-to-day variation in instrument performance. A difference from the certified concentration of more than 10% requires further investigation of that run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the

entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Table 2 lists historical average % recoveries. At least 2 QCS are analyzed on each run.

Standards and reagents are prepared from reagent grade chemicals (typically JT Baker) or from pre-made stock solutions. All glassware is acid washed (10% HCl) and rinsed 6 times with ultra pure-low DOC water (18.2 mega-ohm). All analyses (except CHN) use multi-point calibration curves (4-7) points, which are analyzed at the beginning and the end of each run. A Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB) (a standard run as a sample) and Laboratory Duplicate are analyzed every 10 to 15 samples during each run. At least one Laboratory Fortified Sample Matrix (LFM) is analyzed during each run to insure that sample matrices do not affect method analysis efficiency. Field Duplicates are not required by our lab, and are the responsibility of the specific project's manager.

Laboratory Duplicates must fall within 15% relative percent difference (RPD = abs(dup1-dup2)/average of dup1 and dup 2). A difference greater than 10% requires further investigation of the sample run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for relative % difference are included in Table 2.

LFM must show 85% to 115% recovery. A recovery <90% or > 110% requires further investigation of the sample run. A recovery <85% or >115% is failure (unless the sample is less than 10X the MDL), and results in re-analysis of the entire sample queue,

unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for % recovery are included in Table 2.

Method Detection Limits are calculated at least twice per year, or whenever major changes to instrumentation or methods occur. Table 2 lists most recently measured MDL values.

VIII. Schedule of Internal Audits

Internal audits are not routinely performed, however, review of QC charts, and tables are done at least quarterly by the lab manager.

IX. Preventive maintenance procedures and schedules

The laboratory manager, Jeff Merriam, has 10 years of experience and is highly experienced with all laboratory equipment used within the WQAL. The laboratory manager conducts all maintenance and inspection of equipment based on manufacturer requirements and specifications.

Each day an instrument is used, it receives a general inspection for obvious problems (e.g. worn tubing, syringe plunger tips, leaks). The instruments are used frequently and data is inspected within a few days of sample analysis. This allows instrument (or user) malfunctions to be caught quickly, and corrected as needed.

Each day's run is recorded in the instrument's run log, with the date, the user, the number of injections (standards, samples, and QC samples), the project, and other notes of interests. Maintenance, routine or otherwise, is recorded in the instrument run log, and

includes the date, the person doing the maintenance, what was fixed, and any other notes of interest.

X. Corrective Action Contingencies

Jeffrey Merriam is responsible for all QC checks and performs or supervises all maintenance and troubleshooting. When unacceptable results are obtained (based on within sample analysis batch QC checks) the data from the run are NOT imported into the database. The cause of the problem is determined and corrected, and the samples are re-analyzed. Problems are recorded in the sample queue's data spreadsheet, or on the handwritten runsheet associated with the run. Corrective actions (instrument maintenance and troubleshooting) are documented in each instrument's run log.

XI. Record Keeping Procedures

Protocols, Instrument Logs, QC charts, databases and all raw data files are kept on the lab manager's computer. These are backed up weekly, with the back up stored off site. The computer is password protected, and is only used by the lab manager. Protocols and the sample database are also password protected. Handwritten run sheets are stored in a filing cabinet in the lab. Instrument run and maintenance logs are combined with the QC data to form one large Excel file where instrument performance can easily be compared to instrument repair and the number of analyses, etc. This file is also stored on the lab manager's computer and is password protected.

All information pertinent to a sample is stored in the sample database. From this database we can easily determine the date of analysis and the location of the raw data file

if further review is necessary. The amount of information provided to sample originators is dependent on what is required by the project or funding agencies.

Table 1. List of standard operating procedures and description of analyses done at the Water Quality Analysis Laboratory.

Standard Operating Procedure	Analysis	Instrument Used	Description	Protocol Latest Revision	EPA method or other reference
Ion Chromatography Protocol for Anions and Cations Protocol	Anions and Cations	Ion Chromatograph Ion Chromatograph	Anions via ion chromatography w/ suppressed conductivity. Cations via ion chromatography and conductivity	June 11, 2002	Anions EPA #300.1
Dissolved Organic Carbon Protocol	DOC	Shimadzu TOC 5000 with autosampler	High Temperature Catalytic Oxidation (HTCO)	June 25, 2002	EPA 415.1
Total Dissolved Nitrogen Protocol	TDN	Shimadzu TOC 5000 coupled with an Antek 720 N detector	HTCO with chemiluminescent N detection	June 25, 2002	Merriam et al, 1996
DOC and TDN combined Protocol	DOC and TDN	Shimadzu TOC-V with TNM nitrogen module	HTCO with chemiluminescent N detection	June 25, 2002	EPA 415.1 and Merriam et al, 1996
Lachat QuikChem AE Protocol	Nitrate/Nitrite colorimetric NO_3/NO_2	Lachat QuikChem AE	Automated Cd-Cu reduction	June 25, 2002	EPA 353.2
	Ammonium colorimetric NH_4	Lachat QuikChem AE	Automated Phenate	June 25, 2002	EPA 350.1
	Soluble reactive Phosphorous colorimetric PO_4	Lachat QuikChem AE	Automated Ascorbic acid	June 25, 2002	EPA 365
Acid Washing Protocol	Glass and plastic-ware cleaning		10% HCl rinse and 6 rinses with DDW	June 25, 2002	
Field Filtering Protocol	Sample prep		3-times rinse with filtered sample	June 25, 2002	

Table 2. Detection limits, acceptable ranges, and recent historical averages for QC samples at the Water Quality Analysis Lab.
[1] Detection limit based on user experience and previous analysis (not statistically calculated). [2] Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

Analyte	Units	Typical Range	Regression Type	# of Cal. Points	Detection Limit [1]	MDL [2]	Lab Duplicate % Relative Difference	Limit	LFM % recovery	Limit +/-	IPC % recovery	Limit +/-
SiO_2	mg SiO2/L	0 – 40	Linear	4-7	0.3		3.5	15.0	92.8	15.0		
PO_4	µg P/L	0 – 200	Linear	4-7	2 – 3	1.5	7.8	15.0	95.5	15.0	93.7	15.0
NH_4	µg N/L	0 – 200	Linear	4-7	2 – 3	1.5	7.1	15.0	103.9	15.0	95.0	15.0
NO_3 FIA	mg N/L	0 – 10	Linear	4-7	0.05	0.003	4.6	15.0	100.9	15.0	102.6	15.0
Na^+	mg Na/L	0 – 15	Quadratic	4-7	0.1		0.9	15.0			112.7	
K^+	mg K/L	0 – 7	Quadratic	4-7	0.05		10.4	15.0			97.8	
Mg^{2+}	mg Mg/L	0 – 7	Quadratic	4-7	0.1		4.5	15.0			89.7	
Ca^{2+}	mg Ca/L	0 – 10	Quadratic	4-7	0.1		4.0	15.0			98.2	
Cl^-	mg Cl/L	0 – 15	Quadratic	4-7	0.2	0.02	1.6	15.0			92.7	
NO_3^-	mg N/L	0 – 3	Quadratic	4-7	0.002	0.002	0.3	15.0			96.3	
SO_4^{2-}	mg S/L	0 – 8	Quadratic	4-7	0.1	0.04	2.2	15.0			86.5	
TDN	mg N/L	0 – 10	Linear	4-7	0.1	0.029	7.8	15.0	100.3	15.0	102.1	15.0
DOC	mg C/L	0 – 20	Linear	4-7	0.1	0.048	4.9	15.0	100.5	15.0	97.0	15.0

References

Avanzino R.J. and V.C. Kennedy, 1993. Long-term frozen storage of stream water samples for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research*, 29(10) 3357-3362.

Merriam, J.L, W.H. McDowell, W.S. Currie, 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. *Soil Science Society of America Journal*, 60(4) 1050-1055.

Appendix D-2

Standard Operating Procedures for Lachat Analyses
UNH Water Quality Analysis Laboratory

Lachat Protocol

Instrument overview

All Lachat methods that we use work by measuring a color development brought about by specific reagents. The amount of color is measured via absorbance, which is proportional to the amount of analyte present.

The system is made up of several components. These are described in order from left to right as you look at the instrument.

Autosampler

Holds standards and samples for analysis and has a sampling needle which alternates between being in the sample and being in the rinse vessel. There are 12 lettered vial positions (A – L). These hold the standards in large glass vials. This allows for multiple injections from each standard (typically 2). There are 96 numbered vial positions for samples, blanks, check standards, reference samples, etc.

The sample holders can be removed from autosampler for sample loading. Please do not remove more than three of these holders at one time, as this can cause the autosampler to jam, and requires painful effort to repair.

Pump

A peristaltic pump used to move reagents and sample from their respective reservoirs to the manifold. Pump speed should be 35. Pump tubing should be changed daily to prevent troublesome flow problems. Each pump tube is color coded, indicating its diameter, and thus its flow rate. The manifold diagram found in the specific method you are using will tell you specifically which color to use. The Sample loop, which connects the sampling needle to the manifold should be cut so that 1 inch extends beyond the colored collar on both sides. This ensures accurate timing of sample delivery to the manifold.

Valve

The valve switches sample to and from the sample loop and rinses the loop with carrier. It has six ports. Port 1 and 4 are connected by the sample loop, which supplies a constant volume of sample to be analyzed. The length of the sample loop is specified on the manifold diagram. Port 5 goes to waste from the last channel being used. If you are using two channels, Port 5 on the first channel connects to Port 6 on the second channel. The carrier line connects to Port 2. Port 3 attaches to the manifold.

Manifold

Contains a series of coils, tubes and fittings where the reagents and sample mix and react. There is a heating coil under the manifold that is used when specified in the method. These fittings should be checked periodically to ensure that there are no clogs.

Detector Head

At the end of the manifold is a blue box, which contains the flow cell, the wavelength filter, light source and detector. Be sure that you've installed the correct wavelength filter for the method you are using.

Standard Operating Procedures

1. Select the proper method for the analysis and the sample range you are interested in. Methods are located in two 3-ring binders on the shelf in the lab. The early pages of each method describe the principles of the reaction involved in the analysis, as well as the recipes for the reagents used. There is a manifold diagram (usually toward the end of the method) that describes how the manifold should be set up for each analysis. This description includes the tubing to be used, the length of the sample loop, the wavelength filter, as well as what temperature the heater is set to if needed.

2. Prepare reagents as described in the recipes in the method. Be sure to mix ingredients in the order described as this can affect the outcome of the reagent. Also, pay careful attention to the amounts of acids and bases used in the reagents because many of the color reactions involved in the analyses are pH dependent.

3. Prepare standards as necessary. If you are performing two analyses at once (i.e. running two channels), you should make your standards together (mixed standards). For example, if you were running PO_4 and NH_4, you should have one standard set that contained both NH_4 and PO_4. If you suspect contamination from one standard to the other (i.e. the NH_4Cl used for the NH_4 standard has PO_4 in it), you can make separate standard sets for each analyte. This is normally not an issue.

4. Set up the manifold as specified in the method. Manifolds are found in the cabinet under the lab bench.
   a. Install the correct wavelength filter.
   b. Change pump tubing to appropriate color tubing. Do this daily. Consult the manifold diagram. Be sure the pump tubing is pushed all the way over the nipple on the union before screwing on the fitting. If your fingers don't hurt a little, you probably haven't done it correctly.
   c. Check the manifold for pinched/kinked tubing, plugged unions and T-fittings and unattached tubing. This doesn't have to be done everyday, but taking the 10 minutes required to do this can save you several painful hours troubleshooting later.
   d. Check to make sure the manifold is set up properly by comparing the manifold diagram with the manifold. The sequence that the reagents and samples mix is important and specific for each analysis.

5. If you are generating a hazardous waste, make sure you have enough waste containers on hand before beginning analysis. You will easily go through one or more waste bottles per day. Bottles can be picked up at the Chemistry Stock room in Parsons Hall. See Hazardous Waste protocols for more information on this. The following analyses generate hazardous Waste.

   Ammonium Analysis
   Ortho P and Total P Analysis
   Nitrate/Nitrite Analysis
   Silica

6. Turn the system on by turning the power-strip behind the Lachat on.
7. Stretch pump tubes across the pump cartridge so that the collars are extended beyond the outside edge of the cartridge. Press one side of the cartridge down, and then the other down tightly until you here a light snap, as the cartridge is locked into place. Do this for all the tubes.
8. Turn the pump on, and pump DDW through the manifold and out to waste. Look for any leaks, or pulsing lines. Pulsing indicates some kind of a clog or plug. Fix any flow problems.
9. Check the screen on the Lachat System Unit and note the Diagnostic section on the lower right-hand corner. The instrument reports if there are any problems with the Valves, Detector (alpha only), Reference and Sampler. These should all be "ok" or "OK". Otherwise consult the lab manager.

Computer software use. See attached software flow chart for important components. When you power up the system, the computer should boot into the Lachat software Main Menu. The following describes the different components of the software.

I. **Methods** – Allows you to select a method to load into memory, or to modify or create a method

1. Analysis Select & Download – If the method does not require modification, use this to select and download. You will be put into the **Samples** submenu.
2. Method Definition – Allows you to modify an existing method, or create a new one that meets your analytical needs.
   A. ***File*** – Pick, Save, New, Delete or Quit. Choose the method you want to edit, create, or save, delete a method, or quit from this section.
   B. ***Description*** – Name or Brief notes about the method you selected in the ***File*** menu.
   C. ***Channels*** – Select a channel to modify, add or delete. When you select a channel, you will enter the *Channel Specific Method Definition* (Sheet I.2.C.) screen.
      1. *Info*
         a. *Name of Channel* –
         b. *QuikChem Method Number* – Found on the method sheet used to make reagents.
         c. *Detector Selection* – Ignore. We only have one detector.
      2. *Standards*
         a. *Units* – units of the standards and calculated concentrations.

b. *Format* – format of standards and calculated concentrations.
c. *Concentration* – Enter concentrations of standards for the specific channel. These must be from high to low.

3. *Evaluation*
   a. *Calibration* -- defines the standard curve, and how the software should deal with accepting or rejecting a calibration.
      i. Boundaries – How the standard curve is broken up into high and low ranges, if necessary.
      ii. Strategies – How the software accepts or rejects a calibration. Ignore this unless you're an experienced user.
      iii. Pass/Fail -- Similar to the above. Ignore unless you're an experienced user.
   b. *Signal Processing* -- Ignore
   c. *Auto Dilution Triggers* -- Not Applicable to our system. Ignore.
4. *Presentation* – How the data and peaks are displayed
   a. *Data Window* – Range of the scale and the position of the peak window on the Lachat System display. Ignore unless you're an experienced user.
   b. *Chart Mode* – Ignore.
5. *Timing* – Timing of values and peak detection.
   a. *Periods* – Ignore unless you're an experienced user. You should seek help with this from someone who knows.
   b. *Mode* – Auto or Manual. Ignore this unless you're an experienced user.

D. **Standards** – Allows you to view the current settings and to choose the run protocol for the standards
   1. *Definition* – Read only display of standard concentrations. Ignore.
   2. *Calibration* – Protocols for standard analysis and review.
      a. *Protocol* – How many time each standard gets injected upon doing a calibration (e.g. AA BB CC DD would be standards A through D, each injected twice.).
      b. *Actions* -- Select whether the user must approve all calibrations, or if the software can except it. Chose "User Must Approve" all calibrations.

E. **Timing** – Adjusts system timing. Most timing changes occur in the Channel Specific Method Definition Submenu. Ignore this here unless you're an experienced user.

II. **Samples** – Allows you to start a run, and specify which vials to analyze.
   1. Tray Definition and Submit – Select the appropriate tray to analyze, submit trays, calibrations and kill running trays.
      A. **File** – Select Template Read (public), and pick "temp". This is a sample queue numbered 1 – 96.
      B. **Data Quality Management** – Ignore. We do our own, outside of the Lachat software.

C. **Edit** -- If you have fewer than 96 vials to run, select "Identification", scan down to the last vial in your run, and put a ".." (no quotes) after your last sample. This tells the software to stop sampling.

D. **Submit** – Submit Trays, Calibrations, and kill trays.

1. *Submit Current Tray* – Start a tray. You should answer "yes" when asked if you want to calibrate prior to analysis.
2. *Calibrate Now* -- If you just want to calibrate prior to running for the day to ensure the system is operational, select this.
3. *Kill Running Tray* – Stops the tray currently running.

III. **Results/Approval** – Allows for viewing and downloading of results, and approval of calibrations.

1. View Calibration and Sample Reports

A. **Method Selection** – Select the method used to analyze the data of interest.

B. Tray Selection – Sample tray (*.rps) or Calibration Tray (*.rpc). Runs are saved as the date (YYMMDDrun). For example, 00050801.rps. A sample tray from May 8, 2000, run #01 of the day.

C. Reports – Choose *Report Definition* first and select "runtime" for Sample trays, and "default" for Calibration trays. If you've been shown how to modify a report definition, choose *Define a Report* and make sure you have the correct chord specified. Click *Save Report to Disk*. When the report is prepared, save it to the A: drive.

D. Calibration Graphs and Stats – Allows you to view the calibration graphs in order to approve a calibration. Select the channel of interest. View calibration graph. Further instructions for removing points, etc. are on the screen.

Shut Down

1. Put reagent lines in water. Allow water to pump through manifold for 10 minutes or so. Remember to switch the Cadmium column off line prior to this step if you're doing NO_3/NO_2 analysis.
2. Once water has rinsed all the manifold tubing, pull the reagent lines out of the water so they suck air. Pump until there is little or no water visible in the manifold.
3. Unclip the pump tubing from the pump and release them from the pump cartridges.
4. Clean up your mess, and deal with Hazardous Waste as appropriate.

Notes, things of interest, and specific things

1. NO_3/NO_2 Analysis. The Cadmium column can be ruined easily. To prevent this, keep air OUT of the column. Keep the column out of line, unless you have reagents flowing through the manifold. Remember to switch the column out of line before you flush the manifold with water during shut down.

Quality Assurance and Control

A. Prior to running the Lachat you must log-in on the Log-In Sheet next to the instrument. Please fill-in all designated information. This information will aid in maintenance of the instrument and will be used in conjunction with the Quality Control Table (described later).

B. Every ten to 15 vials (as indicated on the run sheet) will have a blank, a replicate sample, and at least 2 standard replicates.

C. A spiked sample replicate will need to be created for analysis during run as specified on the Lachat run sheet. One spiked sample per run should be generated. The spiked replicate will be made by adding a known volume of 100 mg/L stock solution to 5 mL of sample solution. The weight of each volume should be measured on the balance and recorded. The amount of the spike will depend on the known or anticipated concentration of your samples. 10% to 50% increase in the sample concentration is typically a good rainge.

0.1 mg/L increase = 5 µL 100 mg/L stock in 5 mls
0.5 mg/L increase = 25 µL 100 mg/L stock in 5 mls

If analyzing for multiple constituents (e.g. NH_4 and PO_4 run at the same time) the spiked replicate must contain a known volume of each of these constituents. All volumes should be recorded as weights on the Lachat run sheet.

E. Following completion of your analysis you are responsible for filling out the Quality Control Table located in the main lab filing cabinet. You will need to provide data for your certified reference standards as specified on the Table (see attached Table). This data must be recorded onto the Quality Control Table within one week of sample analysis, failure to do so will result in serious restrictions on laboratory instrument usage. You must also put a copy of your run sheet in the appropriate folder near the Quality Control Tables.

Appendix D-3

Standard Operating Procedures for Total Dissolved Nitrogen Analyses
UNH Water Quality Analysis Laboratory

TDN using HTCO (Antek 721C)

I.) Preparation of Standard Solutions.

NH_4 is typically used to make the standards. You may change this if you feel your samples may present an unusual situation. We have several N-containing chemicals in the lab that you can use to make check standards, or as an alternative to NH_4.

A. Weigh out 0.3819 g of dried NH_4Cl (should be found in the desicator). Dissolve it in a 100 mL volumetric flask, and fill to volume. This makes a 1000 mg N^{L-1} stock solution (1000 ppm). Quantities for other salts are listed below.

Chemical	grams to add to 100 mL to make 1000 ppm
2Na EDTA $2H_2O$	1.3290
NH_4Cl	0.3819
$NaNO_3$	0.6068
Urea	0.2144
Caffeine	0.3466
Glycine	0.5359

B. If the samples to be analyzed are at the lower end of the concentration range, it may be necessary to make an intermediate standard (100 mg $N\ L^{-1}$).

C. Make working standards by pipetting the appropriate amount of stock (or intermediate standard) into 100 mL volumetric flasks, and bring them to volume. You can put the 100 mL volumetric flasks directly on the analytical balance, allowing you to know exactly how much stock you are adding. This eliminates the necessity of weighing water (to determine the volume dispensed) before using the adjustable pipettes.

D. Store stock solutions and standards in their volumetrics in the refrigerator. Stocks will store for up to a month. Standards should be made weekly, or more frequently if dealing with low concentrations (< 0.3 mg/L). Remember to cover tightly with parafilm. **Please remove you stocks and standards from the fridge when you are done. Dump and rinse glassware, acid wash and return cleaned glassware to the appropriate shelf.** Refer to Acid Washing protocol for details.

II. Sample Preparation

A. Sample vials are prepared by rinsing them at least 2 times with DDW and then combusting them in the muffle furnace at 500°C for 6 hours. It takes the muffle furnace one hour to get up to temperature.
B. If you are not sample limited, you should rinse the vials once with sample before filling. Be consistent within a run. If you are sample limited and can not rinse each vial before filling, then do not rinse the standard vials or blanks either.
C. Put a Blank in the first sample position. This sample will NOT be acquired. It is only there so that the instrument and the software (EZ-Chrom) are in sync.
D. Fill each vial to about one (1) inch from the top, and cover with a 1cm X 1cm piece of parafilm.
E. Put vials in the cardboard tray or other holder (not the sample tray).
F. Carry-over is not typically a problem, unless running a low sample (<0.3 mg/L) immediately following a high sample (10 mg/L). As with any analysis, it's best to analyze similar samples together.
G. Please refer to the **Quality Assurance and Control Section** for information on replicates, certified reference standards and check standards. A copy of the TDN Runsheet is attached.

III.) Plumbing and Gas Connections

A. At the bottom of the combustion tube, turn two-way valve to point toward TDN.
B. Leaks usually have something to do with the membrane dryer. If you suspect a leak, check there first.

IV.) System Inspection

A. Confirm TOC-5000 combustion furnace temperature is at 680 C.
B. Confirm gas pressure on the **ultra zero AIR** cylinder. The cylinder pressure should read at least 400 psi. Carrier gas from the tank should be set to 80 psi. Carrier Flow meter (on TOC-5000) should read 250 mL min^{-1}.
C. Confirm gas pressure on the zero grade Oxygen cylinder. The cylinder pressure should read at least 400 psi. Oxygen from the tank to the flow meter should be set to 40 psi. The flowmeter controlling flow to the Antek should be set to between 50 and 100 cc/sec.
D. Allow Oxygen to flow through the Antek for about 10 minutes prior to turning the Antek ON. This flushes the instrument free of nitrogen. Then turn the Antek **ON** (two switches on back-left side of Antek). The Antek may take up to an hour to warm up and perform correctly, so turn it on early if possible.
E. If you smell ozone after you turn the Antek on, you may need to replace the charcoal in the outlet scrubber or you may have a leak. Check charcoal first.
F. Inspect the syringe. The syringe should be clean and operate smoothly. Confirm syringe movement by entering the **Maintenance** menu, **Mechanical Check** screen. Press 1 to move syringe up, press 2 to move syringe down. If

the syringe leaks around the plunger, the plunger can be replaced. See TOC-5000 operator's manual.

V.) Preparation for Analysis

A. It is necessary to do a "Pre-run" before you analyze your samples. This consists of injections of DDW for an hour or 2 before you begin your run. Fill a large Vial with DDW. Cover with parafilm.
    1. Unscrew the sparge and sample needles from the autosampler arm.
    2. Put both needles into the large vial.
    3. Set Vial on the bench top. **NOT** in the Sample Tray.
    4. Start the instrument as you would a normal run (see below)
    5. Prior to sample analysis, it is recommended that you inject a standard to check for instrument response. Put sample needle into standard solution, and allow the instrument to inject the sample. Using the Preview button in EZ-Chrom, you can watch for a response. If no response, there could be a leak or other problem.

B. Check to see that waste vessel is relatively empty, and that the waste tube is in the waste vessel and has no kinks.

C. The TOC-5000 is normally left ON. If it has been turned OFF, turn on the power switches for the Autosampler and the TOC-5000. If on power-up, the TOC-5000 is in the All Reset mode, all calibration files, date, time, etc., have been erased, and these will have to be entered. I have never seen this in over 4 years. If it does occur, consult the TOC-5000 manual.

D. Press the **NEXT** function key to get to the **Main Menu**.

E. Press **3** and **ENTER**, to get to the **General Conditions** screen.
    1. Confirm that the 250 uL syringe is installed. Check this by physically looking at the syringe, and check that the selected syringe **in General Conditions** agrees with this.
    2. Set the printer to OFF. This must be off, or the acquisition of data will be incorrect.
    3. Confirm that the TC Furnace is ON. This is hardly ever turned OFF. If it is, it will take about an hour for the furnace to reach 680° C.
    4. Exit **General Conditions**

F. Enter the Maintenance Screen by pressing **8** and **ENTER**.

    1. Sroll down to **Ready State Sensor** - Turn this to **INACTIVE** by pressing ENTER while it is highlighted.
    2. Scroll down to the **Mechanical Check** selection and press **ENTER**
    3. Fill the wash-bath reservoir (part of autosampler) with fresh water. You can move the sampling arm by pressing the **ASI** button in **the Mechanical Check** screen. Move sampling arm up, and to the left by pressing the **Arm-Up** button and the **Arm-Left** button.
    4. If proceeding with your actual analysis (not the pre-run), screw the **sampling** needle into the sampling arm (Back hole). Sparge needle

does not need to be installed in to the sampling arm, because no sparging is necessary for TDN analysis.

5. If the sampling needles have been removed and re-installed, you may want to see if they are still in line with the vials. Press the V1 button and the sampling arm moves to vial 1 on the tray. Check to see if the needles line up with the vial by pressing the **Arm-Down** key. Press **the Arm-Down** key again to stop the arm. . The V43 button lines the needle up with vial 43.
6. Exit the **ASI** screen and the **Mechanical Check** screen, and return to the **Main Menu**.

G. Press **9** and **ENTER**, to enter the Autosampler screens.

NOTE: After making any changes, remember to press the ENTER key, or your changes will not be saved.

Sample Measurement Conditions screen. Tells the autosampler what injection volume, number of replicates, etc.

-- measurement group number, up to 15 groups. Each line represents a series of samples that will be subject to the same measurement conditions. TOC analysis requires 2 lines, so the maximum number of groups cannot be used. Typically you'll only have one group, unless you perform TOC analysis.

Type -- type of measurement, Select 4=NPOC (Non-Purgeable Organic C) for TDN.

IS -- Initial Sample, specifies vial position in the sample tray of the first sample in a group.

FS -- Final Sample, specifies vial position in the sample tray for the final sample in a group. Must be the greater than or equal to the value of IS.
NOTE: All spaces between the IS and FS sample positions must be filled, or the autosampler will suck air, and your run could suffer.

C1-C3 -- Ignore these settings. These are used if you plan to create and store a calibration file in the software of the TOC-5000. It is not recommended that you use this feature. For more information on this, consult the TOC-5000 manual.

F1-F3 -- Specifies the calibration file already stored in the instrument. Enter **2** here. This sets the TOC-5000 range to 5, and allows you to inject a low volume.

RG -- Range of the detector. The range set in the calibration curve will be entered automatically. You cannot change this setting unless you use a different calibration file. This should be 5 for TDN analysis.

Vol -- Injection Volume. Entered automatically when a curve is specified, but can be changed. Should be set to 15 uL or 20 uL for TDN analysis.

W -- Washes. Sets the number of times the syringe and the sampling and injection lines are rinsed with sample before sample injection begins. This should typically be set to 3 or 4.

No -- Number of injections for each sample. **Set to 4 to TDN**.

Max -- Maximum number of injections per sample. Set this to **4 for TDN**

SD – Ignore

CV -- Ignore

Dil -- Leave this set to 1. We don't have the dilution option.

SP -- Sparge time. Set this to 0 minutes for TDN

H. Press **NEXT** (**F2** key)

ASI Conditions screen.

RINSE -- Sets whether the surface of the sampling and sparging needles will be rinsed in the needle rinse vessel. If '1' is selected, rinsing will be performed before and after each standard and sample. If '2' is selected, rinsing is not performed. If a wide concentration range does not exist between samples, rinsing is most likely not needed.

NO OF NEEDLE WASHES -- Sets the number of times the flow path from sampling needle to injection needle will be rinsed with water (from the rinse water vessel) after sample injection. Set this to 3 for high salt samples to prevent poor injection performance due to a build up of salts on injection needle.

FLOW LINE WASHES -- Number of times the flow lines will be rinsed with rinse water after all analysis are complete. This is especially important for high salt samples. However, leave this set to 4 as routine, to prevent build up of deposits in the flow lines.

CALIBRATE BEFORE -- Ignore this unless you entered a value under C1, C2, or C3 in the previous screen.

PRINT OUTPUT -- Leave this OFF. We are not concerned with what the TOC-5000 calculates for concentrations. Concentrations will be manually determined in a spreadsheet.

AUTO ADDITION OF ACID -- This may only be specified when a NPOC analysis is being performed. If you manually added acid to the samples, turn this OFF.

ACID VOLUME -- Specifies the volume of acid to be added to each vial. Set to 50 uL if you're using the Auto addition option.

RINSE AFTER ADDITION -- Setting this to 'USED' specifies rinsing of the sampling needle in the rinse water vessel after each addition of acid.

KEY LOCK -- Locks the keys while a run is in progress. Leave this "UNLOCKED".

FINNISH OR RUNNING -- **Finnish**, **running**, or **no change** specified here determines events after the completion of all analyses. When **running** is specified, the following items, TC FURNACE, CARRIER GAS and AUTO START TIME are added to the conditions list, and must be set. **Finnish** shuts down the instrument, except for the cooling fan, which remains on until the instrument may be safely turned off. **No change** specifies that the instrument will remain in the operative state after completion of all the measurements. Set this to RUNNING.

TC FURNACE -- Indicates whether or not the TC furnace will remain on. Leave it ON unless you know that no one will use the machine for a week or so.

CARRIER GAS -- Indicates whether or not the carrier gas will keep flowing after analysis is complete. Set this OFF. No sense wasting gas.

AUTO START TIME -- Specifies the time at which the carrier gas or TC furnace will be turned on again.

I. Press **NEXT**.

VI.) Computer overhead Prior to starting Data Acquisition

A.) Accessing EZ-Chrom.

1.) Double click the EZ-Chrom icon in Windows.

2.) Double click **Instrument 2- Antek-TDN.**

3.) You will be asked to provide a user name and a password. A name and password will be provided by the lab manager. See him or her for this info.

B.) Making a Batch File. The batch file is simply the sample queue, listing the samples in the order that you plan to analyze them.

1.) From the menu bar, select **File, New Batch**, and fill in the appropriate information. Appropriate Information includes:

--Method Path. Name of path to be used to find method files.

--Data Path. Where to store the data files.

--Sample ID. The sample's name. It is usually best just to put in something easy here, and edit the batch file later.

--Method Name. Name of method to use to analyze you run. If you need to create a method for you analysis, see the EZ-Chrom manual for details.

--File Name. As with the sample ID, it's easiest to put something easy here and edit the batch file.

--Sample Amount. The amount of each injection (15 or 20, generally).

--ISTD Amount. Leave blank

--Multiplier. If you've performed a dilution on you samples, include the dilution factor here. If not, set this to 1.

--Number of Runs. The number of samples you will analyze. EZ-Chrom calls a sample one run, and a series of samples a batch.

2.) A spreadsheet will appear containing the number of runs and information that you specified above. Now edit the batch as necessary.

--Run Type. This is UNKNOWN for the TDN analysis, as you will do the regression in a spreadsheet later.

--Sample ID. This is just the sample name, typed as you would like to see it in a spreadsheet or report.

--Method. This should read what ever you selected earlier. If not, change it now for all of the samples in your batch.

--Filename. Needs to be a unique name for each sample. This is what you data will be saved as. It must be less than 9 characters long.

--Level. If you aren't including any calibration data, this can be set to zero. If you are including calibration data, this should be filled in with the appropriate level for each standard. You also must change Run Type to Calibration.

--Sample Amount. Set to the injection volume for each respective sample.

--ISTD Amt. Ignore, unless you are using internal standards.

--Mult. Set to one (1) if you don't have any dilutions. If you do, input the dilution factor here.

--Failure Act. Set this to Continue.

--Description. If you want you can type a detailed description of the sample here.

3.) Go to **File, Save Batch As...** and save it. A suggested name would be today's date (e.g. 980330).

VII.) Data Acquisition

At this point, you should have a Method that you want to use (see manual if you need to create or modify one), a Batch File created, and the Antek and TOC-5000 set up correctly and ready to go.

A.) Start TOC-5000 by pressing the START button.

B.) In EZ-Chrom, click the Run Batch button on the tool bar.

1.) Confirm that the Batch Name listed is the correct name.

2.) Confirm that **Start Run #** and **End Run #** are correct. **First** and **Last** are usually okay.

3.) Click the start button. After a few seconds, it should say "Waiting for Trigger" at the bottom of the screen.

C.) After the first sample (which is a blank) EZ-Chrom will start to acquire data.

IX.) Peak Checking

Peak Checking can be done in the Batch Reprocessing Window of EZ-Chrom, or in the Instrument 2 - Antek window. If you want to begin looking at your data before the entire batch is finished, you must go to the Batch Reprocessing window.

A.) Open the Batch file that you wish to check.

B.) From the menu, select **Batch**, **Reprocess**. A dialog box will appear.

1.) **Reprocessing Mode:** Set this to **Reintegrate**.

2.) **Reprocess From:** Set to **First** to **Last**, or the range you want to reprocess.

3.) Put an X in the box next to **Review Results (Pause After Each Run).**
4.) Leave the box next to **Bracket Calibration** empty.
5.) Click **Start** when you're ready to begin.

C.) If your batch contains Calibration files, a dialog box **entitled Calibration Options** will appear. Put an X in the box next to **Clear Response Factors At Batch Start**. Then click **Ok**.

D.) At this point, the chromatograms should be displayed on the screen. **Cntl - Z** normalizes the chromatogram to its highest peak. The baseline can be expanded by clicking and dragging the mouse over the baseline you'd like a better look at. You can return to the normalized view of the chromatogram by using **Cntl - Z**. You can look at the previous image (section of expanded baseline) you viewed by double clicking on the chromatogram.

E.) Select **Method**, **Graphical Events Programming**, **Integration Tools** (or **Cntl-T**). This displays the different Integration tools for changing peak starts and ends and baseline in a button format. These can also be accessed by selecting **Method**, **Graphical Events Programming**, and selecting one from the menu.

The EZ-Chrom manual explains what each of the Integration Tools does. This protocol will give details of the most commonly used tools.

1.) **Manual Baseline (Man BL)**. Draws a new baseline for a peak. Click and drag an new baseline from the start of the peak to the end. This tool allows you to correct the start and end of the peak at the same time. After making this change, a dialog box appears, showing the start and stop time of the change you just made. You may have it reintegrate the chromatogram by clicking that button, or if you have several other changes to make, click **Ok**, which will allow you to make other changes before integrating. It also asks if you want to save changes to the **Manual Integration Fixes Table**, or to the **Integration Table**. Saving to the **Manual Integration Fixes Table** just makes the changes to the current chromatogram. Saving changes to the **Integration Table**, changes the method, and will be applied to each subsequent chromatogram. Generally you'll want to save changes to the **Manual Integration Fixes Table**.

2.) **Start** and **Stop.** Each of these is pretty self explanatory. Changes the start and stop of the peak.

3.) **Integration Off**. Turns off the integration for the selected time range. Can be used when a peak has been detected and identified as one of the peaks of interest, but it isn't the correct peak. Also can be used if there is a two humped peak, and the peak has been split into two peaks by EZ-Chrom. **Integration Off** for one of the peaks, and then use **Manual Baseline** to put in a peak covering both humps.

F.) After you have made all necessary changes to the chromatogram, go to the next chromatogram by typing the down arrow (⇩). If you need to correct a previously viewed chromatogram, you can get back to it by pressing the up arrow (⇧).

G.) After you have checked each of the runs in a batch, you need to make a summary file that you can use in a spreadsheet to calculate concentrations and get you data into a usable form.

1.) Select **Batch**, **Summary** from the menu.

2.) A dialog box will appear. Check the following.

--**Enable Summary for Channel:** A

--**Peak Parameters:** Select those that you are interested in.

--**Group Parameters:** Leave these unchecked.

--**Summary Report Path:** Type in the complete path name where you would like your file saved. EZ-Chrom calls the file Batch.sum, where Batch is the name of you batch file.

--Click OK.

3.) Next, Select **Batch**, **Reprocess** from the menu. A dialog box will appear.

--**Reprocessing Mode:** Set this to **Reintegrate**.

--**Reprocess From:** Set to **First** to **Last**, or the range you want to reprocess.

--make sure **Review Results (Pause After Each Run)** is NOT checked**.**

--Leave the box next to **Bracket Calibration** empty.

--Click **Start** when you're ready to begin.

4.) Open this file in Excel, make changes to it as needed, and save the file. If you want to do your calculations in Quatro-Pro, open it in Excel, and save it as a *.DIF file. Then open this file in Quatro-Pro.

X.) Data Reduction and Number Crunching

This is easily done in the spreadsheet of your choice. The parameters for data reduction presented here are typical, and should be used as a guideline.

A.) Calculate the mean, and %CV of the three peaks for each sample. If the CV is greater than 5 %, use the two peaks closest in area counts to calculate the mean area for that sample.

B.) Because the Blank is generally zero (or very close to zero), use the blank in the calculation of the regression.

X1.) Quality Assurance and Control

A. LOG-IN

Prior to running the ANTEK you must log-in on the Log-In Sheet next to the instrument. Please fill-in all designated information. This information will

aid in maintenance of the instrument and will be used in conjunction with the Quality Control Table to be described later.

B. PRE-RUN

A pre-run should be done prior to analysis. Set the instrument up to inject aliquots of DDW, tap water, or samples from the previous day's TDN run. This should run for several hours prior to your analysis. This is primarily to condition the catalyst.

C. BLANK STABLIZATION

Three blanks will be at the start of your run. The first acts as the "timing blank" (no data is collected for this), and the second is to get a blank value. A third blank is recommended, especially if you're analyzing low TDN (<1 or 2 mg N/L).

D. Standard Replicates, Sample Replicates, Certified Reference Standards

A blank, a sample replicate, and two or three standard replicates will be run every 12 samples as specified on the TDN run sheet. The TDN standard curve frequently drifts during the run, and multiple and frequent replicate standards will allow you to correct for this drift.

1. Sample replicates will consist of one spiked replicate as noted on the TOC run sheet. This replicate is required to check for potential problems due to matrix interference. The spiked replicate will be made by adding a known volume of 1000 mg/L stock solution to 5 mls of a given sample (to be determined by user). If your standard curve ranges from 0 to 10 mg/L or less then you must use a 1 mg/L spike. If your standard curve exceeds 10 mg/L at the high end then you should use a 5 mg/L spike. For calculation of an increase of 1 mg/L above the sample concentration add 5 µL of 1000 mg/L stock to 5 mls of sample.

1 mg/L increase = 5 µL 1000 mg/L stock in 5 mls
5 mg/L increase = 25 µL 1000 mg/L stock in 5 mls

All volumes should be recorded as weights on the TDN run sheet.

2. Two vials will be filled with a certified reference solution (Ultra Scientific, or otherwise) in your run. This should be treated like a sample and will be placed in the positions specified on the TDN run sheet. The Lot # for the certified reference standard should be written on each run sheet. This will allow you to track the run to run variability of your analysis, as well as confirm the accuracy of your standard solutions.

3. At the end of your run, a standard curve consisting of four standards and a blank will be run. This will help to detect and account for any drift in the calibration during the run.

E. QUALITY CONTROL TABLE

Following completion of your analysis you are responsible for filling out the Quality Control Table located in the main lab filing cabinet. You will need to provide data for your EPA certified reference standards as specified on the Table (see attached Table). This data must be recorded onto the Quality Control Table within one week of sample analysis. Failure to do so will result in serious restrictions on laboratory instrument usage. You must also put a copy of your runsheet in the appropriate folder near the Quality Control Tables.

Appendix E
Center for Freshwater Biology Standard Operating Procedures
Field and Lab Sheets

CFB TOTAL PHOSPHORUS ANALYSIS DATA SHEET (200_)

CURRENT DATE:_____ **ANALYZED BY:**_____

Sample ID# / Site Description	a660	a880	TP (ppb)	Spec chk	Transfered
1A 200.0 ppb TP Standard				/	
1B 200.0 ppb TP Standard				/	
2A 40.0 ppb TP Standard				/	
2B 40.0 ppb TP Standard				/	
3A 20.0 ppb TP Standard				/	
3B 20.0 ppb TP Standard				/	
4A 10.0 ppb TP Standard				/	
4B 10.0 ppb TP Standard				/	
5A 2.0 ppb TP Standard				/	
5B 2.0 ppb TP Standard				/	
6A dd H_2O blank				/	
6B dd H_2O blank				/	
7A				/	
7B				/	
8A				/	
8B				/	
9A				/	
9B				/	
10A				/	
10B				/	
11A				/	
11B				/	
12A Lab Spike: 50.0 ppb				/	
12B Lab Spike: 50.0 ppb				/	
13A TP Standard: 20.0 ppb				/	
13B TP Standard: 20.0 ppb				/	
14A				/	
14B				/	
15A				/	
15B				/	
16A				/	
16B				/	
17A				/	
17B				/	
18A				/	
18B				/	
19A				/	
19B				/	
20A				/	
20B				/	
21A				/	
21B				/	
22A				/	
22B				/	

Page Control # - _____

23A TP Standard: 20.0 ppb				/	
23B TP Standard: 20.0 ppb				/	
24A				/	
24B				/	
25A				/	
25B				/	
26A				/	
26B				/	
27A				/	
27B				/	
28A				/	
28B				/	
29A				/	
29B				/	
30A				/	
30B				/	
31A				/	
31B				/	
32A Lab Spike: 50.0 ppb				/	
32B Lab Spike: 50.0 ppb				/	
33A TP Standard: 20.0 ppb				/	
33B TP Standard: 20.0 ppb				/	
34A				/	
34B				/	
35A				/	
35B				/	
36A				/	
36B				/	
37A				/	
37B				/	
38A				/	
38B				/	
39A				/	
39B				/	
40A				/	
40B				/	
41A TP Standard: 20.0 ppb				/	
41B TP Standard 20.0 ppb				/	
42A dd H_2O blank				/	
42B dd H_2O blank				/	

Stock Standard made on (date):_____

Date Chemicals/Standards Made:

5N H_2SO_4_____

11N H_2SO_4_____

10N NaOH_____

Phenolphthalein_____

Potassium antimonyl tartrate_____

Ammonium Molybdate_____

Ascorbic Acid_____

Current TP Coefficients (Based on: _____ - _____ ppb standards)

a880 Phosphorus Coefficient = _____ Calibration curve r^2 = _____

Page Control # - _____

TN Datasheet (200_)

Page Control #_____ **Data Analyst:**_____

Date Processed:_____ **RPD=**

Scan rate = 0.5 nm/second, increment = 0.25nm, path length = 5 cm **Yield=**

Sample	Lake ID	2nd Der.	2nd Der.	2nd Der.	2nd Der.	Average	[TN]
		λ	#1	#2	#3	2nd Der.	(*u* g/L)
1	dd H2O blank						
2	3000 *u* g/L standard						
3	2000 *u* g/L standard						
4	1000 *u* g/L standard						
5	500 *u* g/L standard						
6	250 *u* g/L standard						
7	100 *u* g/L standard						
8							
9	(replicate)						
10							
11	(replicate)						
12							
13	(replicate)						
14	(1664 *u* g/L spike)						
15	(replicate)						
16	1000 *u* g/L standard						
17							
18	(replicate)						
19							
20	(replicate)						
21							
22	(replicate)						
23							
24	(replicate)						
25							
26	(replicate)						
27	1000 *u* g/L standard						
28							
29	(replicate)						

Nitrogen Coefficient:_____ **based on** _____ **to** _____ ***u*** **g/L standards** **r^2:**_____

TN Datasheet (200_)

Page Control #_____ **Data Analyst:**_____

Date Processed:_____

Scan rate = 0.5 nm/second, increment = 0.25nm, path length = 5 cm

Sample	Lake ID	2nd Der.	2nd Der.	2nd Der.	2nd Der.	Average	[TN]
		λ	#1	#2	#3	2nd Der.	(*u* g/L)
30							
31	(replicate)						
32							
33	(replicate)						
34							
35	(replicate)						
36							
37	(replicate)						
38							
39	(replicate)						
40	1664 *u* g/L spike						
41	(replicate)						
42	1000 *u* g/L standard						
43							
44	(replicate)						
45							
46	(replicate)						
47							
48	(replicate)						
49							
50	(replicate)						
51							
52	(replicate)						
53							
54	(replicate)						
55							
56	(replicate)						
56	1000 *u* g/L standard						
57	dd H_2O blank						

Nitrogen Coefficient:_____ **based on** _____ **to** _____ ***u*** **g/L standards** **r^2:**_____

Benthic % Organic Matter Datasheet 200__

Date:__________________ Analyst:____________________ QC:____________

Dish # Dry/Ash	Sample ID (Lake, Site, etc.)	Date	Comments	Drying Dish weight (grams)	Dry weight (grams)	Ash Dish weight (grams)	Ashed weight (grams)	% Organic Matter
/								
/								
/								
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/								
/								
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/								
/								
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/								
/								

Note: Dry Weight and Ashed weights include the weight of the Dis
Analyze 10% of samples as duplicate

Benthic Digestion Sample Tracking 200_

Analyst:_____ **Date:**_____ **QC:**_____

Digest Flask #	TP Flask #	TN Tube #	Dry Weight (grams)	Extract Vol. (ml)	Sample ID (Lake, Site, etc.)	Date	Comments

Batch Run Page ___ 0f ___ page control #: _____ QC Check: _____

Benthic Total Nitrogen Data Worksheet 200_

Analyst: **Date Processed:** **QC: RPD_____** **QC Slope:_____________QC:R-Square:______**

Tube #	Digest Flask	Sample ID (Lake, Site, etc.)	Date	Comments	weight (grams)	volume (ml)	TN mg/L	Dilution Factor	Total Nitrogen (mg/Kg)
1		**DDI Blank**							
2		**3000 µg/L standard**							
3		**2000 µg/L standard**							
4		**1000 µg/L standard**							
5		**500 µg/L standard**							
6		**250 µg/L standard**							
7		**100 µg/L standard**							

TN mg/L from TN page control #__________________

Batch page ___ of ___ Page Control #____________ QC Check:_________

Benthic Total Phosphorus Data Worksheet 200_

Analyst: **Date Processed:** **QC: Slope:____________QC:R-Square:______**

Flask #	Digest Flask	Sample ID (Lake, Site, etc.)	Date	Comments	weight (grams)	volume (ml)	TP (µg/L)	Dilution Factor	Total Phosphorus (mg/Kg)
1A		**DDI Blank**							
1B		**2000 µg/L standard**							
2A		**1000 µg/L standard**							
2B		**500 µg/L standard**							
3A		**100 µg/L standard**							
3B		**50 µg/L standard**							
4A		**10 µg/L standard**							
4B		**5 µg/L standard**							
5A									

TP µg/L from TP page control #___________________

Batch page ___ of ___ Page Control #______________ QC check:____________

Insturment Calibration/Maintenance Log

Instrument: _____

Date	Calibration	Inspection (Specify)	Corrective Action	Acceptance Criteria	Person Responsible (Signature)

CFB DATA LAKE SAMPLING SHEET (200_) DLS #:_____

LAKE:_____ SITE:_____

WHO:_____Time: Start_____ Finish_____

DATE:_____ Zmax:_____meters Zsd:_____meters AirTemp_____ ^{o}C

Depth (meters)	pH	CO_2 (mg/L)	SPCD (υS/cm)	Alk (mg/L)	DTP (ppb)	Chl *a* (ppb)	Diss. O_2 (mg/L)	Color (ptu)	Weather/comments
Surf. 0.5				/					
1/2 epi_____m				/					
1/2 meta____m				/					
Bot-1m_____m				/					
Depth_____m				/					
Depth_____m				/					
Int. 0-_____m				/					

Depth (meters)	Temp (^{o}C)	Diss O_2 (mg/L)	Light deck cell	Light (*uE* /cm)	Light (rel %)
0.1					
0.5					
1.0					
1.5					
2.0					
2.5					
3.0					
3.5					
4.0					
4.5					
5.0					
5.5					
6.0					
6.5					
7.0					
7.5					
8.0					
8.5					
9.0					
9.5					
10.0					
10.5					
11.0					
11.5					
12.0					
12.5					
13.0					
13.5					
14.0					
14.5					
15.0					
15.5					
16.0					

Depth (meters)	Temp (^{o}C)	Diss O_2 (mg/L)	Light deck cell	Light (*uE* /cm)	Light (rel %)
16.5					
17.0					
17.5					
18.0					
18.5					
19.0					
19.5					
20.0					
20.5					
21.0					
21.5					
22.0					
22.5					
23.0					
23.5					
24.0					
24.5					
25.0					
25.5					
26.0					
26.5					
27.0					
27.5					
28.0					
28.5					
29.0					
29.5					
30.0					
30.5					
31.0					
31.5					
32.0					
32.5					

	Depth (meters)	pH	CO_2 (mg/L)	SPCD (υS/cm)	TP (ppb)	Alk (mg/L)	Chl *a* (ppb)	Diss. O_2 (mg/L)	Color (ptu)
Split Sample	Depth_____m					/			
Replicate Sample	Depth_____m					/			

View Scope Comparison Study

WEATHER (circle best descriptor)

Sky: Clear Hazy Cloudy Ovecast

Lake: Calm Ripples Waves Choppy

Wind: Calm Breezy Gusty Windy

Take Secchi Disk reading from the **shady** side of t boat **without** the view sc Secchi Disk Depth (meters	Take Secchi Disk reading from the **shady** side of t boat **with** the view scope Secchi Disk Depth (meters	Take Secchi Disk reading from the **sunny** side of t boat **without** the view sc Secchi Disk Depth (meters	Take Secchi Disk reading from the **sunny** side of the boat **with** the view scope Secchi Disk Depth (meters)
Viewer #1:_____			
1)_____meters	1)_____meters	1)_____meters	1)_____meters
2)_____meters	2)_____meters	2)_____meters	2)_____meters
Viewer #2:_____			
1)_____meters	1)_____meters	1)_____meters	1)_____meters
2)_____meters	2)_____meters	2)_____meters	2)_____meters
Viewer #3:_____			
1)_____meters	1)_____meters	1)_____meters	1)_____meters
2)_____meters	2)_____meters	2)_____meters	2)_____meters
Viewer #4:_____			
1)_____meters	1)_____meters	1)_____meters	1)_____meters
2)_____meters	2)_____meters	2)_____meters	2)_____meters
Viewer #5:_____			
1)_____meters	1)_____meters	1)_____meters	1)_____meters
2)_____meters	2)_____meters	2)_____meters	2)_____meters

Zooplankton

Tow -0____meters

Phytoplankton

Int: 0-____meters
a663___ volume
a750___ _____
Phyto: Y N
a440____ a750____
a493____ a880____

0.5 meters
a663___ volume
a750___ _____
a440____ a750____
a493____ a880____

Meta_____meters
a663___ volume
a750___ _____
Phyto: Y N
a440____ a750____
a493____ a880____

Depth____meters
a663___ volume
a750___ _____
Phyto: Y N
a440____ a750____
a493____ a880____

Depth____meters
a663___ volume
a750___ _____
Phyto: Y N
a440____ a750____
a493____ a880____

Comments (continued from front of sheet):

SPCD Readings (post processing)
_____*u*S Standard = _____*u*S

dd H_2O TP blank _____ ppb

pH Meter Readings (post processing)
pH buffer 7.01 = _____
ph buffer 5.01 = _____
pH buffer 4.01 = _____

Tributary and Shallow Site Documentation

Site	Depth (m)	pH	CO_2 (mg/L)	SPCD (υS/cm)	Alk (mg/L)	DTP (ppb)	Chl *a* (ppb)	Diss. O_2 (mg/L)	Color (ptu)	DLS #

NH LLMP MENDUMS POND STREAM SURVEY DATASHEET

Monitors:_____ Vol or CFB (circle one)
Site (number, name):_____ Date:_____
Sample Depth:_____ Time:_____
Air Temperature:_____ ^{o}C Water Temperature:_____ ^{o}C

Weather: (Check One For SKY and WIND- Use Code Letter For RAIN)

Sky-	Clear_____	Hazy_____	Cloudy_____%	Overcast_____
Wind-	Calm_____	Breezes_____	Gusts_____	Windy_____
Rain-	Current*_____	Past 24 hrs*_____	Past 24-48 hrs*____	Past Week*_____

D=drizzle S=short duration shower L=long duration shower T=heavy storm

Observations (Check All That Apply, Use Bottom and Back For Comments)

Water Appearance

Clear_____	Scum_____	Green_____	Light Brown_____
Milky_____	Foam_____	Orange-Red_____	Dark Brown_____
Tea _____	Muddy_____	Oily Sheen Colored_____	Oily Sheen Dull_____

Stream Bed Coating and Color

None_____	Orange-Red_____	Black_____	Brown_____
Moss_____	Yellow_____	Green_____	Blue-Green_____
Blanket_____	Tufts / Growths _____	Sediments_____	Leaves_____%
	Emergent Plants _____	Woody Debris_____%	

Odor

None _____ Acrid _____ Chlorine _____ Musky _____ Perfume _____
Rotten Egg ____ Sewer/Septic _____ Other* _____

Gauge Readings

____Stream Gauge Reading / ____Water depth in culvert_____inches

Measurements and Samples Taken (_/_ seperates replicate samples)

___ Braided / In Channel: ___ % Part ___ Low ___ Medium ___ Full / ___ Overbank
Clay ____% Silt ____% Sand ____% Gravel ____% Cobble ____% Boulder ____%
(sticky) (fine) (gritty) (0.1"-2.5") (2.6"-10") (>10")
Specific Conductance:_____ /_____*u* S/cm Turbidity_____/_____ ntu
meter #: _____Std. Check:_____µS/cm meter #: _____Std. Check:_____ntu
Dissolved Oxygen_____/_____mg/L pH _____/_____
meter #: _____Std. Check:_____%(air) meter #: _____ Std. Check _____
Total Phosphorus Sample: Y N (circle) TN/Nitrate Sample Y N Frozen? Y N
Date/Time in cooler:_____/_____ Date/Time at lab:_____/_____

Comments/Observations:

Total Mileage _____
Monitor Signature:_____ End Time: _____

TP:_____ppb TN _____ mg/L Nitrate____mg/L Color _____ ptu

Page Control #____-_____ Reviewed:_____

NH LLMP STREAM MONITORING INSTRUCTIONS

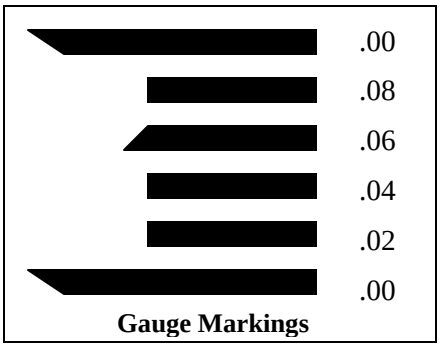
Data Sheet

1- **Fill out data sheet as completely and legibly as possible**
2- **Use a pencil or waterproof ink pen**
3- **Make sure to include all monitors and observers present.**
4- **Include your start time and completion time.**
5- **For OBSERVATIONS use a check mark for all except where percentage cover is asked for (%) or if an asterisk (*) indicates a letter code or written response.**
6- **Report stream gauge readings to the nearest hundredth foot for large gauges.**

Taking the Stream Gauge Reading

1- **Clean any attached material at or just below the waterline of the gauge**
2- **Read gauge where the water level falls if fluctuating select the average height as best you can**
3- **To read the gauge note what the foot number mark (the number with the .0 after it) closest to the water level completely visible is. The foot digit reading is one less than that number. Then note what the closest tenth of a foot number to the water level is. The tenth foot digit is one less than that number.**
4- **For the hundredth foot digit note where the water level hits the horizontal slash marks: Each slash mark is 0.02 feet. The 0.00 mark is the longest with a top point facing left. The 0.02 mark about 2/3 the length of the 0.00 mark, is flat ended, ands falls below a second flat ended mark. The 0.04 mark is the same (flat ended) except it lies just below a bottom pointed mark. The 0.06 mark is that mark and it lies below a flat ended mark (0.08) which falls just below the next 0.00 mark.**

Another way to use the markings- - if you see just above the waterline:
Two short marks both flat ended = 0.00
One short flat ended mark under a short bottom pointed one = 0.02
A short bottom pointed mark = 0.04
A short flat ended mark that lies under a longer top pointed mark = 0.06
A long top pointed mark = 0.08.



Gauge Markings

Have your partner check the reading you made.

Taking a Water Sample

1- **Mark the bottle with site, date, time, organization before taking sample.**
2- **Handle the bottle with care as it contains acid preservative**
3- **Carefully remove the cap being careful to not contact the inside cap or bottle opening.**
4- **Position yourself at a stable area and face upstream.**
5- **Carefully place the bottle under the water pointing upstream without disturbing the stream bottom**
6- **If the water is too shallow look for a deeper pool or sample from a culvert or falls**
7- **Only fill the bottle to the "shoulder" do not overflow!**
8- **Carefully cap the bottle without contacting inside the cap or the opening**
9- **Slowly and gently invert the bottle a few time**
10- **Keep the bottle in a cool dark place until you can freeze it.**

NOTE- The sample preservative is concentrated sulfuric acid. If contact is made flush area with water right away. Use stream or lake water if no other water is available. Keep bottle away from face when opening. Wash hands after use. Gloves and eye protection are recommended.

ADDITIONAL COMMENTS:

Mendums Pond (2006)

Sampling Site:_____ Date:_____

Current Weather (precip):_____ Who:_____

Precip (previous week): _____ Time:_____

Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m			
Stream Width (m) _____(m)	**Stream Depth[1] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[2] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[3] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[4] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[5] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[6] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[7] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[8] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Total Phos. (ppb)** _____ppb	**Temp (°C)** _____°C	**SPCD υS@25°C** _____*u* S

Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m	Horizontal Dist:_____m
Stream Depth[9] (cm) _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[10] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[11] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[12] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[13] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[14] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[15] (cm)** _____(cm) **flow (ft/s)** _____ft/s	**Stream Depth[16] (cm)** _____(cm) **flow (ft/s)** _____ft/s

pH_____

Dissolved Color

a440_____ a493_____

a750_____ a880_____

Color _____CPU

Gauge Height

Take gauge readings to the nearest 0.02 ft

_____ **feet**

Comments/Observations:

CFB Seepage/IPWS Field Data Sheet

Lake:_____ Date: _____ Start Time: _____hrs

Town/State: _____ Site: _____

County: _____ Lake/Site Code: _____

Town Quad: T____N / S R____E / W Sec.____ q:____ Zmax_____ M ft SD _____ M ft

GPS Coord: Lat. _____ Long._____ GMN TRBL Accy/PDOP: _____% Ft M
(circle one) (circle one)

Weather: (Check One For SKY and WIND- Use Code Letter For RAIN)
Sky- Clear_____ Hazy_____ Cloudy_____% Overcast_____
Wind- Calm_____ Breezes_____ Gusts_____ Windy_____
Rain- Current*_____ Past 24 hrs*_____ Past 24-48 hrs*_ Past Week*_____
D=drizzle S=short duration shower L=long duration shower T=heavy storm

Weather / Comments:

Water Appearance: (note all that apply)
Clear_____ Scum_____ Green_____ Light Brown_____
Milky_____ Foam_____ Orange-Red_____ Dark Brown_____
Tea _____ Muddy_____ Oily Sheen Colored_____ Oily Sheen Dull_____

Plant Community Notes (use back of page if necessary):
Stand:_____
Shallows:_____
Deep:_____

Seepage Meter: No.:_____ Condition:_____

Rep 1 Start Volume:_____mls Start Time:_____hrs
End Volume:_____ mls End Time: _____ hrs

Rep 2 Start Volume:_____mls Start Time:_____hrs
End Volume:_____ mls End Time: _____ hrs

Pore Water Nutrient Samples: CL=Co located Samples
Location Relative to Stand Center : Compass Degree_____ Distance_____ Zwater_____ Zpenetration_____
60ml CL1 Bottle Label#:_____ Purge Volume:_____ ml Purge Time:_____min. Fill Time:_____min.
500ml CL1 Bottle Label#:_____ Collected:_____hr Filtered (60ml):_____hr Frozen: _____hr

Location Relative to Stand Center : Compass Degree_____ Distance_____ Zwater_____ Zpentration_____
60ml CL2 Bottle Label#:_____ Purge Volume:_____ ml Purge Time:_____min. Fill Time:_____min.
500ml CL2 Bottle Label#:_____ Collected:_____hr Filtered (60ml):_____hr Frozen: _____hr

Location Relative to Stand Center : Compass Degree_____ Distance_____ Zwater_____ Zpentration_____
60ml CL3 Bottle Label#:_____ Purge Volume:_____ ml Purge Time:_____min. Fill Time:_____min.
500ml CL3 Bottle Label#:_____ Collected:_____hr Filtered (60ml):_____hr Frozen: _____hr

60ml Blank Bottle Label#:_____ 60 ml Dup Bottle Label#:_____CL#____ 60ml bot.blnk Label #_____
500ml Blank Bottle Label#:_____ 500ml Dup Bottle Label#:_____ CL#____ 500ml bot.blnk Label #_____

Sediment Samples: CL1 Bottle Label#:_____ CL2 Bottle Label#:_____ CL3 Bottle Label#:_____
Collected:_____hr Collected:_____hr Collected:_____hr
Dup. Bottle Label#:_____ CL#_____ Frozen: _____hr

Field Personnel:_____

Page Control # 200_ - _____ Reviewed:_____